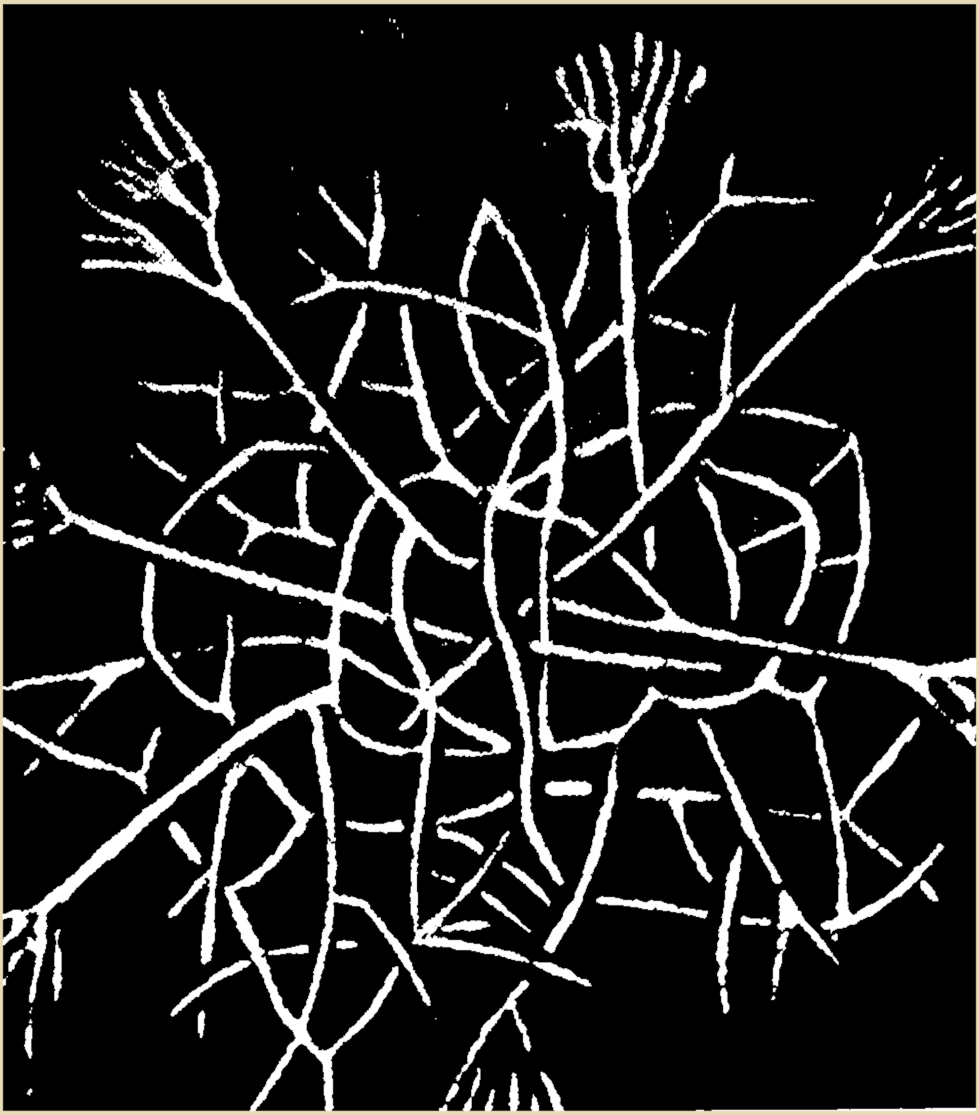
Microbiology



MIR PUBLISHERS MOSCOW

K. PYATKIN

MICROBIOLOGY

In this textbook a systematic, concise and up-to-date account of the main sections of microbiology, virology and immunology is given.

The description of separate causative agents is presented in a certain sequence. First of all, the morphology and structure of microbes are given, followed by data on cultivation, enzymatic properties, antigen structure, classification, etc.

Taxonomy and nomenclature of microorganisms are given in accordance with the International Nomenclature accepted in Soviet periodical and monographic literature.

The morphology of microbes is described using the latest data on the structure of microbial cells obtained with the aid of light and electron microscopy and phase-contrast and luminescent microscopy of ultrathin sections.

The textbook contains new data on the morphology of viruses, on the microflora of the external environment. A section on fungi is systematized.

The textbook is intended for students of medical institutes.

MIR PUBLISHERS

К. ПЯТКИН

МИКРОБИОЛОГИЯ

ИЗДАТЕЛЬСТВО „МЕДИЦИНА“
МОСКВА

На английском языке

K. PYATKIN

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MICROBIOLOGY

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Translated from the Russian

by

L. AKSENOVA and V. LISOVSKAYA

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TO THE READER

Mir Publishers would be glad to have your opinion of the translation and the design of this book. Please send your suggestions to Mir Publishers, 2, Pervy Rizhsky Pereulok, Moscow, USSR.

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PREFACE

Modern medical microbiology has become an extensive science which cannot be fitted into the framework of a textbook, and this makes it quite difficult to compile a teaching guide. During the last few years those sciences which previously represented only small sections of microbiology such as genetics, immunology and virology have developed into independent sciences. In the scope of a textbook for students it is impossible to give complete information on all the causative agents which are known to medical science, and also on comprehensive methods of investigation of bacterial and viral infections.

The second Russian edition of the textbook on microbiology with virology and immunology has been revised in accordance with the curriculum accepted in the Soviet Union. New data from Soviet and foreign literature have been added to the textbook, which to a certain degree supplement many chapters. In the English edition the sections "Pathogenic mycoplasmas" and "L-forms of bacteria" have been introduced. Besides, recent data have been added to those chapters dealing with the theory of the formation of antibodies, the theory of immunity, the mechanism of bacterial recombination (transformation, transduction, conjugation), modern views on the evolution of microorganisms, main properties of the gene and its component parts.

The section on viruses is given in accordance with International Classification. All viruses are subdivided into two classes depending on whether they contain RNA or DNA.

Taking into account the complexity of the problem, that is, to give the main sections in general and special microbiology,

briefly and in an up-to-date form, the author during the preparation of this edition resorted to the help of eminent Soviet microbiologists and epidemiologists. Their valuable consultations and advice helped to eliminate the drawbacks in the manuscript, and they helped with necessary corrections and addenda.

The author does not consider this textbook to meet all the requirements of the higher medical institutions of different countries whose curricula are different from those of the Soviet Union and the socialist countries. However, these differences apply to particular problems, which can be rectified in the process of study.

K. Pyatkin

INTRODUCTION

THE SUBJECT AND PROBLEMS OF MICROBIOLOGY

Microbiology (Gr. *mikros*—small, *bios*—life, *logos*—science) is the science of minute organisms, invisible to the naked eye, named microbes. It is the study of the laws of the life and development of microorganisms, and also of the changes which they bring about in animal and plant organisms and in nonliving matter.

The development of microbiology, as well as other sciences, depends largely on industrial methods, the economic requirements and the general progress of science and engineering. According to the requirements of society, in the second half of the 19th century, microbiology was differentiated into general, agricultural, veterinary and medical microbiology.

Modern medical microbiology has become an extensive science. It is subdivided into *bacteriology*—the science of bacteria, the causative agents of a number of infectious diseases; *virology*—the science of viruses, noncellular living systems capable of causing infectious diseases in man; *mycology*—the study of fungi pathogenic for man, and *protozoology* which deals with pathogenic, unicellular animal organisms. In addition, medical microbiology includes the study of the mechanisms of infection and immunity, the methods of specific therapy and prophylaxis of infectious diseases.

Since the conquest of outer space, space microbiology has been faced with the necessity to study the problems of the biological effects of space radiation, and also the problem of life in outer space and on other planets.

Microbiology as a separate science has special methods of investigation which help in solving a number of important problems regarding public health. These methods are extensively used in the theoretical, and clinical medical sciences.

The second half of the 20th century has been marked by great discoveries in the field of natural science. The use of isotopes, chromatography, spectroscopy, phase-contrast, luminescent and elec-

tron microscopy and modern methods in genetics greatly speeded up the development of medical microbiology and virology, and permitted more detailed investigation into the life of microbes, to reveal the unknown mechanisms of their relation to the environment.

In the 19th century in Russia and in other countries very little attention was paid to the microbiological training of physicians. Separate laboratories, which had been organized on the initiative of progressive-minded men of science, were unable to train medical personnel capable of comprehending the microbiological methods of investigation and employing them skillfully for the treatment and prevention of diseases.

In 1892 G. Gabrichevsky began a lecture course on microbiology at Moscow University. The first department of microbiology in Russia was organized at the Petersburg Women's Medical Institute by D. Zabolotny in 1898.

In conditions provided by socialism, microbiology received unlimited possibilities for its development. At present in the USSR there are more than 80 departments of medical microbiology, more than 50 institutes of vaccines and sera, institutes of virology, epidemiology, microbiology and hygiene. Many of them are world famous research centres.

In the Soviet Union a large network of sanitary-epidemiological, bacteriological and virological stations, and specialized laboratories (antiplague, antibrucellosis, antitularaemic, etc.) has been founded which provided for the organizational, methodological, research and practical work in the prevention and eradication of infectious diseases.

Due to the victory of socialism, and the constant concern of the Communist Party and Soviet government for the health of the working people, Soviet Public Health Service has been most successful. In the USSR smallpox, cholera, plague, epidemic relapsing and typhus fever, dracunculosis (rishta), chancroid, malaria, and pappataci fever have been eradicated. Only isolated cases of enteric fever, brucellosis, diphtheria, poliomyelitis, anthrax, etc., have been recorded in some regions of the country.

A SHORT HISTORICAL OUTLINE ON THE DEVELOPMENT OF MICROBIOLOGY

Long before the discovery of microorganisms certain processes caused by their life activities, such as fermentation of wine juice, milk, yeast, etc., were known to mankind. In ancient times at the beginning of civilization, man by using these processes learned to prepare koumiss, sour milk and other products.

In the early stages of the development of science, physicians and naturalists tried to learn the causes of infectious diseases. In the works of Hippocrates (460-377 B.C.), Verona (2nd century B.C.), Lucretius (95-55 B.C.), Pliny (23-79 A.C.), Galen (131-211 A.C.) and other great learned men of that time, the hypothesis of the living nature (*contagium vivum*) of contagious diseases was stated.

The peoples of Asia had certain ideas on the contagiousness of some diseases, and isolated those suffering from leprosy. Avicenna (980-1037) thought that all infectious diseases were caused by minute living creatures, invisible to the naked eye and transmitted through air and water.

The epoch of feudalism was characterized by poor development of productive forces, a low standard of agricultural machinery, a specific town trade and the oppression of the church damping any signs of scientific ideas. In Europe throughout the Middle Ages epidemic diseases rampaged—leprosy, smallpox, plague, typhus fever, etc. Infectious diseases were especially widespread in the countries of Europe and the Middle East during the Crusades. People had erroneous ideas as to the causes of infectious diseases, the routes of their transmission, and methods of combating them. Theologians, referring to the "fathers of the church", whose authority was considered to be infallible, demanded absolute recognition of their absurd notions of the phenomena in nature. Only with great difficulty and selfless work, persecuted by the church, the progressive minds of that period step by step laid the way for science based on experiments.

Microbiology in the Period of Development of Commercial and Industrial Capital. The Invention of the Microscope. The Investigations of A. Leeuwenhoek

With the development of physics, chemistry and medicine during the Renaissance and the period of industrial revolution in the 16-18th centuries, observations and scientific investigations concerning the essence of infectious diseases began to accumulate in Western Europe and in Russia (H. Fracastorius, 1478-1553; T. Sidenham, 1624-1689; D. Samoilovich, 1744-1805 et al). At the beginning of the 17th century due to the success attained in the field of optics, the previously unknown invisible mysterious world of minute organisms was discovered.

In 1590 glass polishers Hans and Zaccharius Jansen constructed a device with magnifying glasses which permitted them to see minute objects. In 1609-1610 G. Galileo (1564-1642) made the first simple microscope. In 1617-1619 the first double-lense microscope with a single convex objective and ocular appeared, the inventor of which was thought to be the physicist C. Drebbel. This microscope was used to study the cells of plant and animal tissues,

and also minute living organisms (M. Malpighi, J. Swammerdam, A. Leeuwenhoek, A. Kircher, R. Heuke et al).

The first person to see and describe microbes was a Dutch scientist, A. Leeuwenhoek (1632-1723). He himself made simple lenses which magnified 160-300-fold. In 1678, A. Leeuwenhoek published his letters on "animalicula viva", live animalcules, which he observed in water, various infusions, faeces, and teeth scrapings. In 1695, he published his work *The Secrets of Nature Discovered by Anton Leeuwenhoek*. A. Leeuwenhoek undoubtedly not only discovered microbes, but also accurately drew them. According to his letters to the London Scientific Society, he actually carried out observations on different microorganisms.

The discovery by A. Leeuwenhoek stimulated a live interest among scientists, and became the starting point for the study of the microworld. However, for some length of time scientists were unable to use these wonderful investigations for learning the causes of fermentation, putrefaction and infectious diseases. More than 150 years passed before the search for causative agents of infectious diseases was successfully completed.

The Nature of Infectious Diseases and Methods of Their Prevention. Investigations by D. Samoilovich and E. Jenner

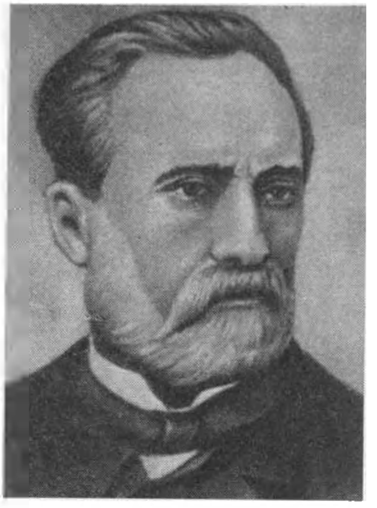
With the development of microbiology, attempts were made to apply this science to the practical problems being faced in the battle against epidemic diseases.

The Russian physician, D. Samoilovich, contrary to the idealistic views predominant in that age, courageously stated the idea that plague is caused by a special and particular substance. He came to the conclusion that to avert the plague an attenuated infectious material should be introduced into the organism. To prove this he dauntlessly carried out a dangerous experiment. In 1771 he inoculated himself with infectious material taken from a man recuperating from the bubonic plague. For his profound study of the problems of combating plague, D. Samoilovich was nominated honorary member of many West European academies.

The statements made by D. Samoilovich in relation to the cause of infectious diseases played an important part in the further development of theoretical and practical problems of the prophylaxis of plague and many other infectious diseases.

In 1798 the English physician E. Jenner (1749-1823) published his wonderful observations on the results of vaccinations against smallpox. He proved that vaccination of humans with cowpox protects them from infection with smallpox. This discovery by E. Jenner armed medicine with a mighty weapon for the successful combat of this disease (see p. 494).

Advances in Microbiology in the Second Half of the 19th Century



L. Pasteur (1822-1895)

With the development of industrial capitalism providing for the rapid growth of natural science and technical sciences, microbiology began to develop quickly. Already in the first half of the 19th century some microorganisms, causative agents of infectious diseases, were discovered. In 1839 J. Schoenlein established that favus is caused by a pathogenic fungus. In 1843 D. Gruby revealed the causative agent of trichophytosis (ringworm). In 1849-1854 A. Pollender, C. Davaine, F. Brauell discovered the anthrax bacillus.

In the second half of the 19th century better microscopes appeared which improved the methods of microscopy. During the study of microorganisms attention was paid to the biochemical processes—the ability of microbes to ferment organic substances.

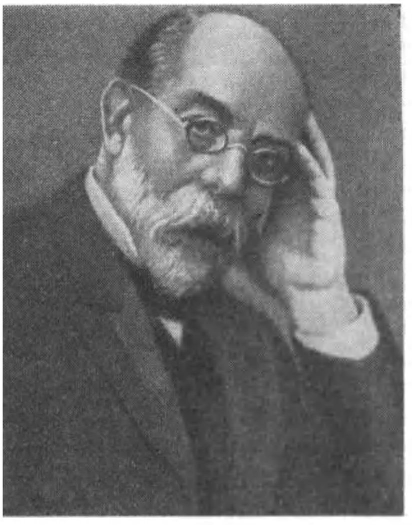
The name of the great French scientist, chemist and microbiologist L. Pasteur (1822-1895) is linked with the most important discoveries in the field of microbiology.

L. Pasteur brilliantly fulfilled the prediction of the physicist and philosopher of the 17th century, R. Boyle, that the nature of contagious diseases would be understood only by a person who could explain the nature of fermentation. L. Pasteur proved the microbial nature of alcoholic (1860), lactic acid and butyric acid fermentations (1861). He demonstrated a new anaerobic type of respiration in some microbes. He discovered that putrefaction is caused by the activity of certain species of microorganisms.

Of great importance are the works of L. Pasteur on the diseases of wine and beer, silkworms (pebrine), and the measures of their control. The data he obtained served as a foundation for industrial microbiology. The investigations of L. Pasteur on the causative agents of chicken cholera, anthrax, and rabies formed a basis for the use of protective inoculations.

In his investigations L. Pasteur did not overlook the problem of spontaneous generation, around which a battle raged in the second half of the 19th century no less furious than in the 18th century.

In 1860 the French Academy of Science paying much attention



R. Koch (1843-1910)

to this problem issued a prize to the person who could solve it. From his observations that microbes are ubiquitous, L. Pasteur devised a method for protecting nutrient media from the penetration of microbes. He proved that spontaneous generation of living substances does not exist. However, it is necessary to note that L. Pasteur's denial of the possibility of spontaneous generation of microbes at this stage of development of our planet is not in the least controversial towards theories of the generation of life from non-living matter in the far past.

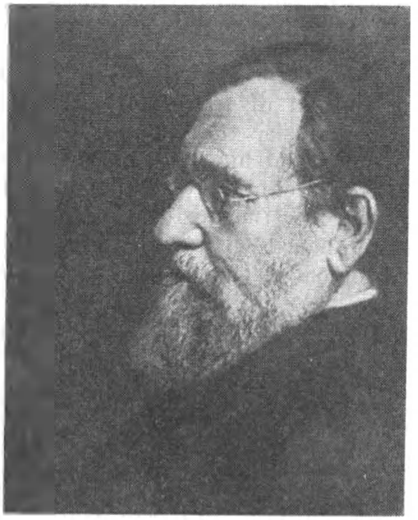
The works of L. Pasteur became the basis for the development of medical microbiology. They drew the attention of many scientists to the study of important problems and encouraged this new science to flourish.

Due to the discoveries made by L. Pasteur, surgery was enriched with improved methods of combating suppurative processes in wounds. The English surgeon J. Lister introduced into surgery the principle of antiseptics (disinfection of wounds with chemical disinfectants).

Improvement of the Methods of Investigating Microorganisms and the Further Development of Microbiology

Of great importance in the progress of medical microbiology were the discoveries made by the German scientist, R. Koch (1843-1910), whose improved methods of investigation contributed to the advancement of microbiology. He and his pupils introduced solid nutrient media (potatoes, gelatine, coagulated serum, meat-peptone agar), aniline dyes, the immersion system, the Abbé's condenser and microphotography into laboratory technique. Due to the improvement of the techniques and methods of microbiological investigations, R. Koch finally established the aetiology of anthrax (1876), discovered the causative agents of tuberculosis (1882), cholera (1883), and obtained tuberculin from tubercle bacilli (see p. 432).

R. Koch made a detailed investigation of wound infections and worked out a method for isolating pure cultures of pathogenic bacteria. He developed a large school of microbiology and among his pupils were K. Eberth, G. Gaffky, E. Klebs, F. Loeffler, S. Kitasato, and many others.



E. Metchnikoff (1845-1916)

The Problem of Immunity to Infectious Diseases. Specific Therapy and Prophylaxis of Infectious Diseases

The successes of medical microbiology in the field of the aetiology of infectious diseases naturally determined the necessity of studying the mechanism of defense reactions—immunity. In working out this very important theoretical and practical problem, a great contribution was made by the outstanding Russian scientist E. Metchnikoff (1845-1916), the founder of the study of immunity of the organism to infectious diseases.

The classical works of E. Metchnikoff on the biological theory of immunity marked a new stage in the development of medicine. As a result of long-term investigations E. Metchnikoff discovered and studied the process of intracellular digestion in some animals brought about by mesodermal cells. His experiment in 1882 on the transparent larvae of starfish showed that the mesodermal cells protect the body of these animals from injections of foreign matter. These investigations served the basis for proposing that similar cells (leucocytes, cells of the spleen and bone marrow, etc.) possess a defense mechanism against pathogenic microbes which have penetrated into the bodies of man and animals. The mesodermal cells were named phagocytes by E. Metchnikoff. The phagocytic theory of immunity was expounded in 1883 at the VII Congress of Russian naturalists and physicians in Odessa.

The study of phagocytosis became the basis for the understanding of the processes of inflammation. E. Metchnikoff showed that inflammation is an active reaction against pathogenic microbes, which provides for the defense of the body, obtained during the process of evolutionary development of animals and man.

E. Metchnikoff paid much attention to determining the causes of early aging, and to the struggle for human longevity. He laid

the foundations for the study of microbial antagonism which later was applied in the production of antibiotics. E. Metchnikoff together with the French microbiologist, E. Roux, in 1903 worked out a method of reproducing experimental syphilis. He also studied tuberculosis, the nature of malignant tumours, and many other problems of biology and medicine.

E. Metchnikoff organized the first Russian bacteriological station in Odessa (the building remains to this day and bears on its façade a signboard with the inscription: "Here was housed the first bacteriological station in Russia founded by E. Metchnikoff, 1886-1888").

E. Metchnikoff's scientific and social activity aroused the suspicion of the Tsarist government. A world-renowned scientist at the height of fame, he was forced to leave his native land. For 28 years (1888-1916) until the end of his life he worked in Paris at the Pasteur Institute. The list of his works containing 322 titles rhetorically speaks of the scientist's creative activity.

E. Metchnikoff was a brilliant spokesman on behalf of Russian scientific ideas, a patriot who indefatigably fought for the priority of Russian science. While in Paris, he remained a Russian citizen, and often visited Russia for scientific purposes. He always helped in the development of his country's science and founded a large school of Russian microbiologists (G. Gabrichevsky, A. Bezredka, I. Savchenko, L. Tarasevich, N. Gamaleia, D. Zabolotny, N. and F. Chistoviches and many others).

E. Metchnikoff's study of the problems of phagocytosis was the starting point for the appearance of a number of works which demonstrated that in the defense reactions of the body an important role is played by certain substances in the blood serum, secreted by special cells under the influence of microbes and their toxins (P. Ehrlich, R. Pfeiffer, J. Bordet, etc.).

In 1888 the French scientists E. Roux and A. Yersin established that the causative agent of diphtheria produces a biological toxin, and they determined its importance in the development of the disease. In 1890 the German scientist, E. Behring, and the Japanese, S. Kitasato, by means of successive injections of small doses of tetanus and diphtheria toxin into animals, prepared the corresponding immune sera which were able to protect the animals from lethal poisonings with toxins. At the Pasteur Institute, E. Roux obtained an antidiphtheric serum and used it for treating diphtheria in children. In Russia the antidiphtheric serum was prepared by G. Gabrichevsky in 1894.

These discoveries served the basis for the production of medicinal sera against botulism, gas gangrene, venomous snake bite, etc.

The German scientist P. Ehrlich (1854-1915) created the theory of humoral immunity from which arose a prolonged difference of opinion dividing the scientists into two schools: supporters of P. Ehrlich and his opponents headed by E. Metchnikoff. The controversy

stimulated a rapid series of investigations of the problems of immunity, the results of which were of great practical importance. More improved laboratory diagnostic methods of infectious diseases were devised, and vaccines were obtained against enteric fever, cholera, plague and other diseases. Due to widespread discussions, it was established that insusceptibility to infectious diseases depends on cellular as well as humoral immunity. The scientists who discovered the causative agents of a number of infectious diseases (Table 1) have performed great services for the advancement of medical microbiology.

In the 20th century very important investigations were made in the field of specific prophylaxis of infectious diseases. In 1924-1925, G. Ramon devised a method for the preparation of anatoxins (toxins rendered harmless by formalin). With their help immunization against diphtheria and tetanus was successfully carried out. Vaccine preparations were received from live, but attenuated, causative agents of tuberculosis (A. Calmette and C. Guérin, 1919), plague (C. Girard and J. Robic, 1931), yellow fever (M. Taylor, 1936), tularaemia (N. Gaisky, 1939), poliomyelitis (A. Sabin, 1954-1958) and a number of other vaccines.

Investigations which permitted the practical introduction of salvarsan (P. Ehrlich), bacteriophages (F. D'Herelle), sulphonamides (G. Domagk et al), penicillin (A. Fleming, E. Chain, H. Florey), streptomycin (S. Waksman, A. Schatz, E. Bugie) and other antibiotics are highly appraised and it is due to the application of these drugs that modern medicine achieved great success in the treatment of infectious diseases.

The development in the last ten years of the genetics of microorganisms and viruses, as a result of which the biochemical mechanisms of heredity and variations were revealed, should be considered a new stage in microbiology. The genetics of bacteria and viruses is of great importance in the origin of a new field of science—molecular biology. (J. Lederberg, E. Tatum, J. Bidl and others).

The success of microbiology contributed to the development of the study of infectious diseases, epidemiology, virology, immunology, surgery, hygiene, etc. It can be said without exaggerating that at present there is no medical science which could have progressed without the development of microbiology.

DEVELOPMENT OF MICROBIOLOGY IN RUSSIA AND THE USSR

At the beginning of the 18th century Russia embarked on the path of rapid economic progress. The reforms of Peter I eliminated restrictions on the development of trade and industry. Peter I was interested in the applied sciences, and attempted to develop them in Russia. While in Delft, Holland, in 1698, he met A. Leeuwenhoek

Table 1

Dates of the Discoveries of the Causative Agents of the Most Important Infectious Diseases

Years	Authors	Microorganisms
1839	J. Schoenlein	Causative agent of favus
1843	D. Gruby	Causative agent of trichophytosis
1849-1854	A. Pollender, C. Davaine, F. Brauell	*Bacillus anthracis*
1859	D. Lambl	Lamblias
1868-1873	O. Obermeier	*Borrelia recurrentis*
1873-1874	A. Hansen	*Mycobacterium leprae*
1874-1885	T. Bilroth, L. Pasteur, O. Ogston, F. Fehleisen, F. Rosenbach, C. Chamberland, A. Weichselbaum	Pathogenic streptococcus
1875-1903	F. Leish, F. Schaudinn	*Entamoeba histolytica*
1877-1916	L. Pasteur, J. Joubert, R Koch, G. Nuttal, F. Novy, M. Weinberg, C. Seguin	Causative agent of gas gangrene
1878-1884	R. Koch, L. Pasteur, A. Ogston, F. Rosenbach	Pathogenic staphylococcus
1879	A. Neisser	Gonococcus
1880	K. Ebert, G. Gaffky	*Salmonella typhosa*
1880	A. Laveran	Malarial plasmodium
1882	R. Koch	*Mycobacterium tuberculosis*
1882	K. Fritsch, N. Volkovich	Causative agent of rhinoscleroma
1882	F. Loeffler, F. Schutz	Causative agent of glanders
1883	R. Koch	*Cholera vibrio*
1883-1884	E. Klebs, F. Loeffler	*Corynebacterium diphtheriae*
1884	F. Rosenbach	Causative agent of erysipeloid
1884	A. Nicolaier	Causative agent of tetanus
1885	T. Escherich	Colibacillus
1885-1898	D. Salmon, A. Gaertner, K. Kensche, G. Nobele	Causative agents of food toxinfections
1885	A. Fraenkel	Causative agent of lobar pneumonia
1885-1899	P. Ferrari, O. Petersen, A. Ducrey, P. Unna	Bacillus of soft chancre
1886-1914	D. Bruce, B. Bang, G. Traum	Brucella
1887	A. Weichselbaum	Meningococcus
1887	K. Harz	Causative agent of actinomycosis
1888	K. Friedlander	Bacillus of pneumonia
1888	V. Carter	Causative agent of sodoku (rat-bite fever)
1891-1892	M. Afanasiev, R. Pfeiffer	Bacillius of influenza
1891-1898	A. Chantemesse, K. Schiga	Bacteria of dysentery
1900-1917	S. Flexner, K. Sonne, K. Schmitz, M. Stutzer	Bacteria of dysentery
1892-1906	G. Guarnieri, E. Paschen	Virus of smallpox
1893	R. Abel	Bacillus of ozaena
1894	A. Yersin, S. Kitasato	*Pasteurella pestis*

Table 1 (continued)

Years	Authors	Microorganisms
1896	C. Ashard, R. Bansod, G. Schottmueller, A. Brion	Salmonella of paratyphoids A and B
1896	E. van Ermengem	Clostridia of botulism
1897	F. Loeffler, P. Frosch	Foot-and-mouth disease virus
1898-1903	B. Babesch, A. Negri	Intracellular inclusions in rabies (Babesch-Negri bodies)
1898-1903	P. Borovsky, W. Leishmann	Leishmania
1901	W. Reed	Virus of yellow fever
1902	G. Dutton, G. Stevens	Causative agent of sleeping sickness
1904-1913	R. Ross, E. Junkovsky	Borrelia of tick-borne relapsing fever
1905	F. Schaudinn, E. Hoffmann	*Treponema pallidum*
1906	J. Bordet, O. Gengou	Bacillus of whooping cough
1908	C. Nicolle, L. Manseau	Toxoplasm
1908-1909	K. Landsteiner, E. Popper	Virus of poliomyelitis
1910-1916	G. Ricketts, C. Prowazek	Rickettsiae of typhus fever
1911-1917	Aragao, E. Paschen	Virus of chickenpox
1912	G. McCoy, C. Chapin	Causative agent of tularaemia
1912	A. Whitmore, K. Krishnaswami	Causative agent of melioidosis
1914-1915	R. Inado, Y. Ido	Causative agent oi icteric leptospirosis
1915-1917	F. Twort, F. d'Herelle	Bacteriophages
1926	E. Murray	Listeria
1933	W. Smith, K. Andrewes, P. Laidlaw	Virus of influenza
1933	K. Meyer	Causative agent of ornithosis
1934	C. Johnson, E. Goodpasture	Virus of parotitis
1934-1938	M. Hyashi, A. Smorodintsev, A. Shubladze	Virus of Japanese encephalitis
1937	L. Zilber, M. Chumakov, V. Soloviev, E. Levkovich	Virus of tick-borne encephalitis
1938	H. Plotz	Virus of measles
1940-1946	A. Smorodintsev, M. Chumakov	Viruses of haemorrhagic fevers
1942-1962	G. Findlay, F. McCallum, W. Raitsell	Virus of epidemic hepatitis
1944	M. Eaton and oth.	Causative agent of atypical pneumonia
1948-1956	G. Doldorf, G. Sickles, J. Enders, G. Melnick and oth.	Coxsackie and ECHO viruses
1953	W. Row and oth.	Adenoviruses
1957	S. Stewart, B. Eddy	Virus of polyoma

who acquainted him with his microscope, showed him the blood flow in the capillaries, and demonstrated a number of microscopic objects.

In the workshops at the Russian Academy of Science, achromatic microscopes were being made with the help of which scientists studied the world of minute organisms.

The growth of natural science is indebted to M. Lomonosov (1711-1765) who was the first to use a microscope when studying the problems of mineralogy and chemistry.

The development of microbiology in Russia is associated with the names of the famous physician and scientist D. Samoilovich and also the outstanding experimental biologist, physician and microbiologist M. Terekhovsky (1740-1796).

In his fundamental work, M. Terekhovsky established that heat sterilizes nutrient substrates. He maintained that organisms in general and microorganisms in particular are not produced by any creative force but conform to a law common to all animals and descend from their predecessor parents by reproduction.

A great contribution to microbiology was made by L. Tsenkovsky (1822-1887), the contemporary of L. Pasteur and E. Metchnikoff. In his investigations he proved the relationship between the plant and animal worlds. He was the first to show the similarity of bacteria and blue-green algae. In 1883 L. Tsenkovsky obtained a highly effective, stable and harmless vaccine which for more than 60 years has been used in Russia for the prevention of anthrax in farm animals. L. Tsenkovsky established the formation of a sugar gum under the influence of certain bacteria (*Leuconostoc mesenteroides*), and devised a new method of inhibiting this harmful phenomenon in the sugar industry.

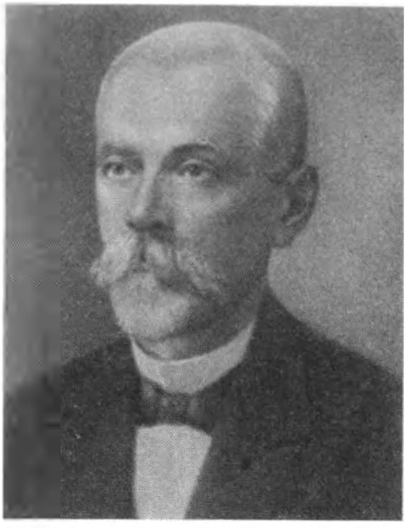
The end of the 19th century saw the rise of agricultural microbiology, the founder of which was S. Vinogradsky (1856-1953). His investigations can be truly placed among the most important achievements of agricultural science and economy. In 1890 he discovered nitrifying bacteria, and studied their importance in the nitrogen cycle in nature (see p. 120). Simultaneously with a Dutch physician M. Beijerinck he discovered nitrogen-fixing bacteria.

The investigations of Russian scientists were extremely significant in the development of medical protozoology. In 1875 F. Lesh (1840-1903) observed dysentery amoeba in the faeces of a patient which were identified by F. Schaudinn as *Entamoeba histolytica*. In 1898 P. Borovsky (1863-1932) discovered the causative agent of skin leishmaniasis (see p. 553).

Russian science laid the foundations for the study of a new field in biology, the study of viruses, presented by D. Ivanovsky (1864-1920). In 1892 while working in the Nikitsky Botanical Gardens on the problem of tobacco mosaic disease which had caused great damage to tobacco plantations, D. Ivanovsky established that this disease, widespread in the Crimea and Caucasus, is produced by a virus which has a high virulence and a strictly selective activity. The discovery by D. Ivanovsky showed that organisms exist together with the cellular forms, which are invisible in ordinary light microscopes. They pass through filters of small pore size and lackacellular structure.

Within 6 years after this discovery by D. Ivanovsky M. Beijerinck confirmed the data obtained by the Russian scientist. Due to the outstanding investigations of D. Ivanovsky, F. Loeffler and

P. Frosch in 1897 the virus aetiology of foot-and-mouth disease was established. Later on, the causative agents of many virus infections of man, animals and plants were discovered and studied.



D. Ivanovsky (1864-1920)

In 1886, L. Pasteur's contemporary and a close colleague of E. Metchnikoff, N. Gamaleia (1859-1949), suggested that bovine plague is caused by a virus. In 1898 he observed the dissolution of microbes which, as established by F. D'Herelle, was caused by the activity of a phage (bacterial virus).

Under the supervision of E. Metchnikoff, N. Gamaleia participated in the establishment of the first Russian bacteriological station in Odessa, and the second Pasteur station in the world. His investigations were dedicated to the study of infections and immunity, bacterial variations, prophylaxis of typhus fever, smallpox, plague and other diseases.

The development of Russian microbiology was beneficial towards the progress of the study of infectious diseases. Microbiological methods were widely used in the study of problems concerning the localization of causative agents in the organism, the routes of transmission of epidemic diseases, and methods of preventing them.

Great services in the development of the study of infectious diseases have been rendered by many Russian scientists, selfless fighters for the maintenance of human health, whose wonderful discoveries have been characterized by a humane and social attitude, heroism, and great examples of sacrifice in science as well as in practical medical activities.

Great heroism was shown by I. Deminsky, M. Lebedeva, V. Turchinovich-Vizhnikevich, I. Mamontov, M. Shreiber, A. Berlin and others, who were infected during their work on the problem of plague control, and died from this infection.

G. Minkh and O. Mochutkovsky in experiments on themselves established the infectivity of relapsing and typhus fever, and came to the correct conclusion that these diseases are transmitted by blood-sucking insects.

The founder of the Moscow school of microbiology, G. Gabrichevsky (1860-1907) investigated scarlet fever, diphtheria, plague, and

N. Gamaleia (1859-1949)

spirochaetal infections. He organized in Moscow the production of an antidiphtheric serum, and was the first in the world to obtain an antiscarlatinal vaccine.

Well merited fame surrounds the name of D. Zabolotny (1866-1929) who together with V. Visokovich studied the epidemiology of plague. The former demonstrated the therapeutic effect of antiplague serum, the mechanism of acquired immunity, and scientifically substantiated the epidemiological role of marmots in the development of natural foci of plague. He established the lytic effect of the serum of syphilitic patients on treponema.

The outstanding scientists include: E. Martsinovsky, who dedicated his investigations to the problems of combating malaria, leishmaniasis, tick-borne relapsing fever, brucellosis, mosquito fever; V. Visokovich, who proved the active participation of the connective tissue cells in defense reactions of the body; L. Tarasevich, who studied the mechanism of the effect of enzymes of phagocytes, the effectiveness of preventive inoculations against tuberculosis, methods of combating typhus fever and other diseases; and I. Savchenko, who investigated the problem of immunity to anthrax, cholera and relapsing fever, obtained an antiscarlatinal serum, and took an active part in installating measures for epidemic disease prevention.

These names by no means exhaust the list of well known Russian microbiologists. It includes many other scientists who enriched the science of microbiology, and the practice of combating infectious diseases.

When speaking of medical microbiology, one must mention the contribution made by Soviet scientists of whom Soviet Public Health Services are justly proud and who attained great success in the control of infectious diseases.

Due to the scientific utilization of the methods of early diagnosis, the active recognition of the sick, their hospitalization, effective decontamination of the environment, and widespread use of inoculations, in the USSR cholera was eradicated in 1926.

Smallpox was liquidated in 1936 owing to compulsory smallpox vaccination, extensive research and practical work carried out on

a scientific level, and improvement in the production and control of the smallpox vaccine.

During World War II epidemiological service provided for the successful prophylaxis of infectious diseases on the battle-front as well as behind the lines. In this respect medical microbiology has performed great services.

By 1960 malaria which once was widespread was eradicated throughout the whole territory of the Soviet Union.

Soviet scientists have revealed infectious diseases unknown earlier (e.g., tick-borne encephalitis, rickettsioses transmitted by ticks, haemorrhagic fevers) and elaborated effective measures for their prevention. The foci of other diseases transmitted through the agency of insects and ticks have been studied. Vaccines for tularaemia, epidemic parotitis, measles, and influenza have been produced. New drugs and methods of laboratory investigation and a number of other discoveries which have advanced world microbiology have been introduced into practice. The names of scientists who contributed to microbiology are found in the corresponding sections of the textbook.

PART ONE

*

GENERAL MICROBIOLOGY

Translated by V. Lisovskaya

CLASSIFICATION AND MORPHOLOGY OF MICROORGANISMS

CLASSIFICATION OF MICROBES

Microorganisms constitute a very antique group of living organisms which appeared on the Earth's surface almost 1,500 million years ago. The questions arising from the study of the origin and evolution of microorganisms are extremely complex. Some scientists assumed that microbes were the first living organisms of the Earth. Others maintained that noncellular forms of living matter (archebionts, photobionts, protobionts, etc.) appeared prior to the microbes. It is now generally regarded that organisms evolved along the following lines: viruses containing RNA, viruses containing DNA, chlamydias, rickettsiae, mycoplasmas, bacteria, blue-green algae, lower and higher fungi, plants and animals.

Aspects relating to the origin of pathogenic microorganisms will be discussed in the chapter "Infection and Infectious Processes".

Medical microbiology is mainly concerned with the study of pathogenic bacteria, actinomycetes, spirochaetes, rickettsiae, viruses, fungi and common protozoa all grouped under the name of microbes or microorganisms.

The great majority of microbes are invisible to the naked eye. They comprise multicellular organisms (the blue-green algae, some fungi and chlamydobacteria, possibly some corynebacteria, mycobacteria, cocci), unicellular organisms (bacteria, actinomycetes, spirochaetes, and protozoa), and noncellular organisms (the rickettsiae and viruses).

The great Swedish naturalist C. Linnaeus, who had never used a microscope, was unable to identify the numerous and diverse forms in the minute world of microorganisms, and classified all the microbes into one genus, giving it the original name of Chaos.

The first attempts to classify microorganisms were based solely on morphological properties. The Danish naturalist, O. Müller (1786) subdivided the bacteria into two genera, *Monas* and *Vibrio*.

In 1827 the Russian zoologist A. Lovetsky, while studying different organisms identified three genera of microbes: *Bacillus*, *Vibrio* and *Proteus*, and described a number of other bacteria.

In 1838 the German scientist C. Ehrenberg subdivided the microorganisms into bacteria, vibrios, spirilla and spirochaetes.

The German botanist F. Cohn in 1854 classified all bacteria as plants. In 1871 he contributed to the knowledge of microbe taxonomy by adding the genera: micrococci, bacteria, bacilli, vibrios, spirilla and spirochaetes.

In 1856 L. Tsenkovsky, the father of Russian microbiology, pointed out the similarity between bacteria and blue-green algae, and thus scientifically based the classification of microorganisms in respect to their evolution.

The German botanist C. Nägeli in 1857 combined all bacteria into a special group of plant microorganisms—the class *Schizomycetes*.

Investigations during the morphological period of the development of microbiology were dominated by a descriptive approach, and microbes were studied without considering their evolution, variations and relation to environmental conditions. As a result, the investigations in microbiology could not be used in agriculture, industry and medicine.

A considerable amount of facts concerning the different properties of microorganisms was accumulated during the 19th century, the list of microbe species gradually increased, and a need for classifying them arose. However, even at present this problem has not been completely solved.

K. Lehmann and R. Niemann in 1896 laid the foundations for a scientific approach to the classification of microbes, according to which all microorganisms were subdivided into three families: *Coccaceae*, *Bacteriaceac*, *Spirillaceae*. Eventually this classification was considerably altered, and was supplemented to a large degree, but even this could not satisfy microbiologists, and it was gradually abandoned in favour of more complete and modern classifications (D. Bergey, N. Krasilnikov et al).

The definition of a species has always presented a difficult but important problem in the classification of microorganisms, and many definitions of a species have been proposed.

On the basis of modern practice a microbial species is used to designate a group of related organisms which: (a) have ascended from a common ancestor, (b) are kept intact by factors of selection, (c) are adapted to a particular environment, (d) are similar in manner of metabolism and character of interspecies relations, and (e) are closely related morphologically, physiologically, and genetically. For pathogenic species of bacteria the ability to produce certain defense substances, antibodies, in the body of animals and man is taken into account (see p. 212).

Probably, in the identification of an unknown species of great importance will be a constant criterion based on the ratio of paired nitrogenous bases in the DNA of bacteria:

$$\frac{\text{guanine} + \text{cytosine}}{\text{adenine} + \text{thymine}}$$

In *Clostridium perfringens* this ratio is equal to 0.48, in strepto-

cocci—0.51, yeasts—0.56, hay bacillus—0.74, colibacillus—1.09, diphtheria bacilli—1.20, tubercle bacilli—2.08, sarcinae—2.57, actinomycetes—2.73, etc.

In order to decide to which species the microorganism under investigation should belong, it is necessary first of all to establish its main properties and then to identify it by these properties, thereafter determining its position in the classification of microbes according to a key.

In microbiology the binominal system of nomenclature is accepted where each species has a generic and a specific name. The generic name is written with a capital letter, and the specific name —with a small letter. For example, the golden pus coccus is called *Staphylococcus aureus*; the anthrax bacillus, *Bacillus anthracis*; the diphtheria bacillus, *Corynebacterium diphtheriae*; the tubercle bacillus, *Mycobacterium tuberculosis*; the tetanus bacillus, *Clostridium tetani*, etc.

If there is some doubt as to whether a special characteristic has been inherited by the culture under investigation, it is known as *a variant*. Variants are those microorganisms which differ from the type species by some slight variations. The term *strain* designates a microbial culture obtained from the bodies of humans or animals and from the environment.

A mixed culture consists of more than one species of microorganism isolated from a natural medium (nonsterile body cavities, body tissues, food products, water, air, soil, washings). *Pure cultures* represent a single species of a particular microorganism.

Following the rapid development of microbial genetics and selection, the term *clone* was applied in microbiology to designate a group of individuals arising from one cell.

The system most widespread in medical microbiology is the International Classification founded by an American bacteriologist, D. Bergey. His manual in its seventh edition (1957) includes descriptions of more than 1,500 species of microbes (there are more than 3,000 known species of bacteria). All lower organisms of the plant kingdom, *Protophyta*, are subdivided into three classes:

Class I: *Schizophyceae* (blue-green algae)
Class II: *Schizomycetes* (microorganisms lacking chlorophyll)
Class III: *Microtatobiotes* (rickettsiae, viruses, filterable microbes)

Class I, *Schizophyceae*, is comprised of microorganisms embracing a large group of blue-green algae which do not contain species pathogenic for man and animals.

Class II, *Schizomycetes*, includes 10 orders 5 of which (II, III, VI, VII, VIII) are nonpathogenic for man and warm-blooded animals. Certain families and genera of the orders I, IV, V, IX, X contain species pathogenic for man and animals.

Class II. *Schizomycetes*

1. Order *Pseudomonadales* (motile cells, with polar flagellation, and nonmotile cells)

   7. Family *Spirillaceae*
      1. Genus *Vibrio*
      7. Genus *Spirillum*

2. Order *Chlamydobacteriales* (filamentous bacteria, motile and nonmotile)

3. Order *Hyphomicrobiales* (bacteria multiplying mainly by budding)

4. Order *Eubacteriales* (true bacteria, rod-shaped, coccal-shaped, motile with flagella arranged peritrichously, and nonmotile)

   4. Family *Enterobacteriaceae*
      1. Genus *Escherichia*
      2. Genus *Aerobacter*
      3. Genus *Klebsiella*
      8. Genus *Proteus*
      9. Genus *Salmonella*
      10. Genus *Shigella*
   5. Family *Brucellaceae*
      1. Genus *Pasteurella*
      2. Genus *Francisella*
      3. Genus *Bordetella*
      4. Genus *Brucella*
      5. Genus *Haemophilus*
      6. Genus *Actinobacillus*
   6. Family *Bacteroidaceae*
      1. Genus *Bacteroides*
      2. Genus *Fusobacterium*
   7. Family *Micrococcaceae*
      1. Genus *Micrococcus*
      2. Genus *Staphylococcus*
      3. Genus *Gaffkya*
      4. Genus *Sarcina*
      6. Genus *Peptococcus*
   8. Family *Neisseriaceae*
      1. Genus *Neisseria*
   10. Family *Lactobacillaceae*
      1. Genus *Diplococcus*
      2. Genus *Streptococcus*
      5. Genus *Peptostreptococcus*
   12. Family *Corynebacteriaceae*
      1. Genus *Corynebacterium*
      2. Genus *Listeria*
      3. Genus *Erysipelothrix*
   13. Family *Bacillaceae*
      1. Genus *Bacillus*
      2. Genus *Clostridium*

5. Order *Actinomycetales* (actinomycetes—filamentous, branching cells)

   1. Family *Mycobacteriaceae*
      1. Genus *Mycobacterium*
   2. Family *Actinomycetaceae*
      1. Genus *Nocardia*
      2. Genus *Actinomyces*
   3. Family *Streptomycetaceae*

6. Order *Caryophanales* (large, nonmotile, segmented cells, joined in filaments).

7. Order *Beggiatoales* (cells occur singly, in filaments, sometimes spherical-shaped, sulphur globules are found in cells).

8. Order *Myxobacteriales* (slime bacteria, rod-shaped, nonrigid cells, motile, nonflagellate).

9. Order *Spirochaetales* (spirochaetes and treponemas, cytoplasm in the form of spirals with at least one turn about a slender axial filament, nonrigid, nonmotile).

1. Family *Spirochaetaceae*
2. Family *Treponemataceae*
   1. Genus *Borrelia*
   2. Genus *Treponema*
   3. Genus *Leptospira*

10. Order *Mycoplasmatales* (small, pleomorphic, filterable microorganisms, pathogenic species cause pleuropneumonia in cattle, agalactia in goats and sheep, atypical pneumonia in humans, etc.).

Class III. *Microtatobiotes*

1. Order *Rickettsiales*

1. Family *Rickettsiaceae*
   1. Genus *Rickettsia*
   2. Genus *Coxiella*
2. Family *Chlamydiaceae*
   1. Genus *Chlamydia*
   2. Genus *Miyagawanella*
3. Family *Bartonellaceae*
   1. Genus *Bartonella*
4. Family *Anaplasmataceae*
   1. Genus *Anaplasma*

2. Type viruses (Vira) are divided into two subtypes: subtype Deoxyvira which includes viruses containing DNA, and subtype Ribovira which embraces viruses containing RNA.

The new classification of viruses was discussed at the Ninth International Congress of Microbiology. This classification is based on the type of nucleic acid (DNA or RNA), anatomy of the viruses, type of symmetry (spiral, cuboid, binary), the number of capsomers in the viruses with a cuboid symmetry, and on the presence of an external membrane.

All the viruses known to the present time (more than 3,000) are grouped into 5 classes, 8 orders, 21 families. The families are composed of genera, which in their turn are made up of species designated in Latin, according to the binominal principle.

Viruses containing ribonucleic acid (RNA)

Mixoviruses: viruses of influenza, parainfluenza, epidemic parotitis, measles, rabies.

Picornaviruses: virus of epidemic poliomyelitis. Coxackie and ECHO viruses, rhinoviruses, viruses of foot-and-mouth disease, and infectious hepatitis.

Arboviruses: viruses of tick-borne encephalitis, Japanese encephalitis, St. Louis encephalitis, haemorrhagic fevers, yellow fever, Dengue fever, sandfly fever, and others.

Viruses containing desoxyribonucleic acid (DNA)

Poxviruses: viruses of smallpox and others.

Herpes viruses: viruses of human herpes, chickenpox, and herpes zoster.

Adenoviruses.

Viruses of tumours and leukoses.

The above mentioned classification which is presented here in an abbreviated form, although containing faults, is the most widely recognized. In this book the names of microorganisms will be given according to the International Rules of Nomenclature.

This classification does not include fungi and unicellular *Protozoa* which belong to independent phyla and are studied separately from bacteria, actinomycetes, spirochaetes, rickettsiae and viruses.

MORPHOLOGY AND STRUCTURE OF BACTERIA

Bacteria (Gr. *bacteria*—rod) are unicellular organisms which lack chlorophyll. Because of their biological properties and methods of reproduction, predominantly by binary fission, they belong to the class *Schizomycetes*, order *Eubacteriales*.

The unit of bacterial measurement is the micron, which is equivalent to 1/1,000 of a millimetre. The dimensions of bacteria vary from 0.1μ for *Mycoplasma laidlawii*, the smallest bacteria, to 2 to 3 by 15 to 20μ for *Spirillum volutans* and even up to 1 mm for the giant sulphur bacteria. The majority of the pathogenic bacteria have dimensions in the order of 0.2 to 10μ.

The shape of spherical bacteria represents a certain ratio of surface area ($As=4\pi r^2$) to volume ($Vs=\frac{4}{3}\pi r^3$). For those cells having a proper cylindrical shape the formulae will be: $At=2\pi b(b+2a)$; $Vi=2\pi ab^2$, where a is equal to one-half the maximum length, b is equal to one-half the maximum width, and r is equal to the radius of the spherical cell.

The shape as well as the dimensions of microbes is not absolutely constant. Morphological differences are found in many bacterial species. The organisms are subject to change with the surrounding environmental conditions. However, in relatively stable conditions, the microbes are capable of retaining their specific properties (size, shape) inherited during the process of evolution.

Morphologically, bacteria possess three main forms. They are either spherical (cocci), rod-shaped (bacteria, bacilli and clostridia) or spiral-shaped (vibrios and spirilla).

Cocci (Lat. *coccus*—spherical). These forms of bacteria (Fig. 1) are spherical, ellipsoidal, bean-shaped, and lanceolate. Cocci are subdivided into six groups according to cell arrangement, cell division and biological properties.

1. *Micrococci* (*Micrococcus*). The cells are arranged singly or irregularly. They are saprophytes, and live in water and in air (*M. agilis*, *M. roseus*, *M. luteus*, etc.).

2. *Diplococci* (Gr. *diplos*—double) divide in one plane and remain attached in pairs. These include: pneumococcus, causative agent of lobar pneumonia, meningococcus, causative agent of epidemic cerebrospinal meningitis, and gonococcus, causative agent of gonorrhoea and blennorrhoea.

3. *Streptococci* (Gr. *streptos*—twisted) divide in one plane and are arranged in chains of different length. Some streptococci are pathogenic for humans and are responsible for various diseases.

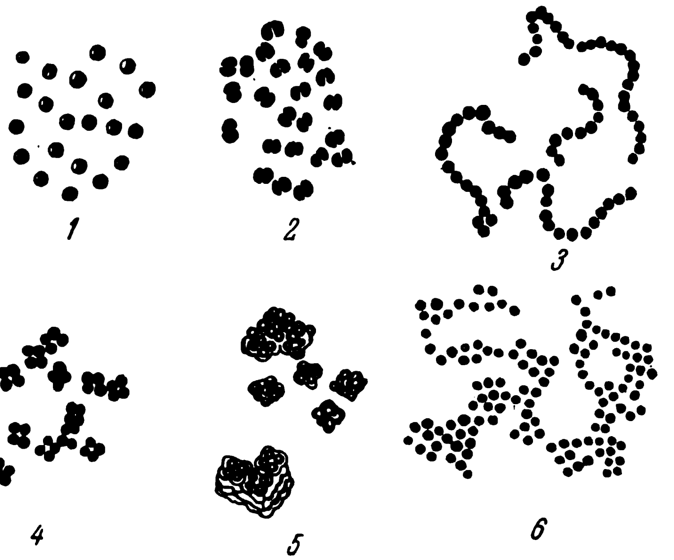


Fig. 1. Spherical forms of bacteria

1—micrococci; 2—diplococci; 3—streptococci; 4—tetracocci; 5—sarcinae; 6—staphylococci

4. *Tetracocci* (Gr. *tetra*—four) divide in two planes at right angles to one another and form groups of fours. They very rarely produce diseases in humans.

5. *Sarcinae* (Lat. *sarcio*—to tie) divide in three planes at right angles to one another and resemble packets of 8, 16 or more cells. They are frequently found in the air. Virulent species have not been encountered.

6. *Staphylococci* (Gr. *staphyle*—cluster of grapes) divide in several planes resulting in irregular bunches of cells, sometimes resembling clusters of grapes. Some species of staphylococci cause diseases in man and animals.

Rods. Rod-shaped or cylindrical forms (Fig. 2) are subdivided into bacteria, bacilli and clostridia. Bacteria include those microorganisms which, as a rule, do not produce spores (colibacillus, and organisms responsible for enteric fever, paratyphoids, dysentery, diphtheria, tuberculosis, etc.). Bacilli and clostridia include organisms the majority of which produce spores (hay bacillus, bacilli responsible for anthrax, tetanus, gas gangrene, etc.).

Rod-shaped bacteria exhibit differences in form. Some are short (tularaemia bacillus), others are long (anthrax bacillus), the majority have blunted ends, and others have tapered ends (fusobacteria).

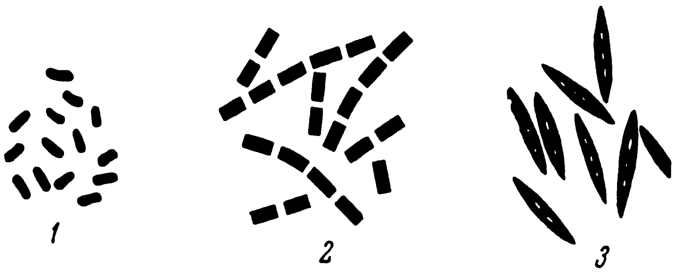


Fig. 2. Rod-shaped bacteria

1, 3—nonsporulating bacteria; *2*—sporulating bacteria

According to their arrangement, cylindrical forms can be subdivided into three groups: (1) *diplobacteria* and *diplobacilli* occurring in pairs (bacteria of pneumonia); (2) *streptobacteria* or *streptobacilli* occurring in chains of different length (causative agents of chancroid, anthrax); (3) *bacteria* and *bacilli* which do not arrange themselves in a regular pattern (these comprise the majority of the rod-shaped forms).

Some rod-shaped bacteria have pin-head thickenings at the ends (causative agents of diphtheria); others form lateral branchings (bacilli of tuberculosis and leprosy).

There is a significantly greater number of rod-shaped bacteria than coccal-shaped organisms. This is explained by the fact that in rod-shaped bacteria the ratio of surface area to volume is higher. Thus, a larger surface area is in direct contact with nutrient substances in the surrounding environment.

Spiral-shaped bacteria. Vibriones and spirilla belong to this group of bacteria (Fig. 3).

1. *Vibriones* (Lat. —*vibrio*) are cells which resemble a comma in appearance. Typical representatives of this group are *Vibrio comma*, the causative agent of cholera, and aquatic vibriones which are widely distributed in fresh water reservoirs.

Fig. 3. Spiral-shaped bacteria

1—vibriones; *2*—spirilla

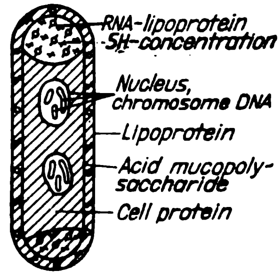


Fig. 4. Structure of *Escherichia coli*

2. *Spirilla* (Lat. *spira*—twist) are coiled forms of bacteria exhibiting twists with one or more turns. Only one pathogenic species is known (*Spirillum minus*) which is responsible for a disease in humans transmitted through the bite of rats and other rodents (rat-bite fever, sodoku).

* * *

Microbes exhibit pleomorphism, they are subject to individual variations, unassociated with age or stage of development, causing the existence of different forms of cells in the same species. They are extremely labile, and susceptible to changes which are associated with such factors as temperature, nutrients, salt concentration, acidity, metabolic products, disinfectants, drugs and body resistance.

Long-term microbiological investigations have proved that the properties observed in successive generations of microbes growing in a fresh favourable medium differ from the properties established for the original generations of a particular species.

Bacteria frequently display pleomorphism particularly when cultivated on artificial media. In response to the chemical and physical properties of the nutrient medium, pathological cells of different size and shape are produced. They may be greatly enlarged, swollen, spherical, conical or filamentous in shape or occur as filterable forms. The changes occurring in the bacterial cell depend on the strength and intensity of the influencing factors, which may affect the genotype (hereditary) or phenotype (nonhereditary). The bacterial genotype is the composition of genetic determinants which determine the whole complex of inherited characters. Phenotype is the complex of characters observed in bacteria under particular environmental conditions. Therefore, the phenotype of bacteria represents the result of the interaction between its genotype and environment.

The ability of microbes to form variant under the influence of different environmental factors reflects the universal law of the development of all forms of living matter. Since microorganisms are subject to change, this is taken into account in laboratory diagnosis of infectious diseases, and when preparing biological preparations used for prophylaxis and treatment. This problem will be examined in detail in the chapter "Genetics of Microorganisms".

* * *

Structurally bacteria resemble the cells of plants and animals and consist of cytoplasm, a nucleus and a wall.

Despite its rather simple structure, a bacterial cell represents a complex living organism (Fig. 4).

The structure of bacteria can be examined with the aid of electron microscopy and microchemical methods which have been highly developed during the past few years, and are employed in identifying the structural and cellular constituents of the microorganism with a high degree of accuracy.

The cytoplasm of bacteria consists of a dispersed colloid of water, proteins, carbohydrates, lipids, mineral compounds and other substances. When the culture ages, the dispersion of the cellular cytoplasm becomes granular, and encloses vacuoles containing cell sap.

The bacterial cytoplasm contains minute granules 100 to 200 Å in diameter (1 angstrem is equal to 0.0001μ). These granules consist of ribonucleoproteins known as ribosomes which are a site of protein synthesis (p.79). Numerous *inclusions* are located in the cytoplasm, comprising volutin granules, lipoprotein bodies, glycogen, granulose, accumulations of pigment, sulphur, calcium, etc.

Volutin granules which contain metaphosphate stain more intensively than the cytoplasm. They are very electron dense. In size the volutin granules vary from several hundreds of Å to 0.5μ.

A characteristic feature of the granules of volutin is their metachromatic stain. With methylene blue they are stained reddish-purple, while the cytoplasm is stained blue.

Volutin was first discovered in the cell of *Spirillum volutans* (from which it was named), then in *Corynebacterium diphtheriae* (Fig. 5) and other organisms. The presence of volutin is taken into account in laboratory diagnosis of diphtheria.

Lipoprotein bodies. These are found quite frequently as droplets of fat in certain bacilli and spirilla. They disappear when the cells are deprived of nutrients, and appear when bacteria are grown on nutrient media of a high carbohydrate content. They are discernible, if stained with Sudan or fuchsin.

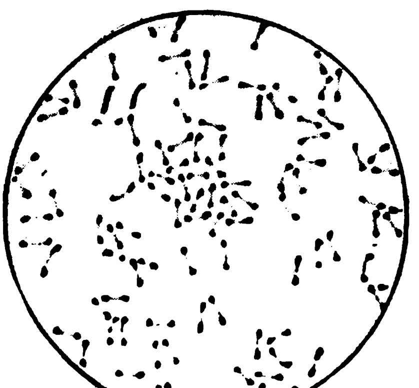


Fig. 5. Granules of volutin in *Corynebacterium diphtheriae*

The presence of volutin granules and lipoprotein bodies is biologically important since they serve as sources of stored food for the bacterium in the case of starvation.

Glycogen and *granulose* are intracellular inclusions, which can be identified by treating the cell with Lugol's solution. Glycogen stains reddish-brown and granulose, grey-blue. Glycogen granules are prominent in aerobic bacilli. Granulose is frequently found in butyric-acid bacteria, and especially in *Clostridium pectinovorum*.

Some bacteria contain crystals of a protein nature, which have proved to be extremely toxic for certain insect larvae.

In the cytoplasm of sulphur bacteria (*Beggiatoa*) which oxidize hydrogen sulphide, *sulphur* is deposited in the form of droplets of a colloidal nature. Energy derived from the sulphur is utilized in reducing carbon dioxide.

Granules of amorphic calcium carbonate, the physiological function of which is not yet known, are found in the cytoplasm of some sulphur bacteria (*Achromatium*).

The cytoplasm of bacteria contains vacuoles which consist of various substances dissolved in water and enclosed by a membrane (tonoplast) of a lipoprotein nature. The number of vacuoles in the cell varies from 6 to 10, and during the period of active growth increases to 20. The biological function of vacuoles is as yet unknown. Some investigators consider them to be regions for depositing toxic metabolic products, others suggest that they function as sources of supplementary enzymatic activity in respiration. It is possible that vacuoles are formed when a large amount of water accumulates in the cell.

Electron microscopy studies of the bacterial cytoplasm have revealed *mitochondria*, possessing a membrane, an oxidizing and reducing enzyme system, and containing phospholipids and sometimes metaphosphates. They vary in size. Mitochondria occur in those parts of the cytoplasm where oxidation-reduction processes take place. Their presence has been shown in colibacilli, in some species of mycobacteria, salmonellae, etc.

During the division of certain bacteria a number of authors have demonstrated convoluted membranes in the transverse septa at the periphery of the cytoplasm (Fig. 6), which have been named *mesosomes*. Enzymatic systems which carry electrons are found in the mesosome membrane. It has been suggested that mesosomes play an important role in cell wall formation and during division.

Fig. 6. Mesosome of the bacterial cell

Nucleus. Bacteria have an undifferentiated nucleus which functions in the life processes, multiplication and sporulation. The nucleus is in close contact with the cell body and its functional manifestations in the environment.

Some authors ascertain that the bacterial nucleus is diffuse, others consider the bacteria to have morphologically distinct nuclei or chromosomes. However, this is a controversial subject and the structure of the bacterial nucleus has not been definitely ascertained. Very detailed contributions by many authors on nuclear material have been accumulated. So far it has been revealed that the nucleus of the bacterial cell is located in the centre, and is represented by a thick strand of chromatin (Fig. 7). However, it has been proposed that during different stages of development of the

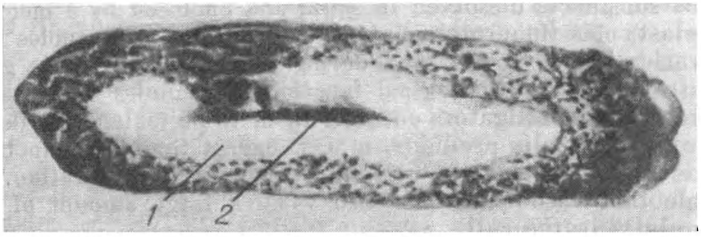


Fig. 7. Ultrathin section of an *Aerobacter aerogenes* cell
1—nuclear vacuole; 2—thick strand

microbial cell, the nuclear material may be diffused throughout the cytoplasm or may be present in a definite form.

Up to the present time the questions concerning the nature of bacterial division (whether it is amitotic or mitotic) remain unanswered. It has been established that the colibacillus may have either a diploid or haploid form of nucleus.

The bacterial nucleus in actively developing cells has a refractive index similar to that of the cytoplasm. The ratio of nuclear material to the cytoplasm varies from 1:2 to 1:10. The nuclear substance can be identified by Robinow's and Feulgen's microchemical test for detecting desoxyribonucleic acid (DNA). By this method *the ribonucleic acid* (RNA) contained in the cytoplasm can be differentiated from *the desoxyribonucleic acid* (DNA) in the nucleus. The nuclear material can be isolated by extracting the nucleic acids with a 5 per cent trichloroacetic acid solution at a temperature of 90°C. The nucleus consists mainly of DNA fibrillae measuring from 20 to 50 Å in diameter. Despite the fact that the nucleus differs chemically from the cytoplasm, these cellular structures are closely interrelated.

The **bacterial wall** consists of a cytoplasmic membrane and a cell wall. In some species, the bacterial cells are surrounded by a capsule.

The cytoplasmic membrane lies immediately beneath the inner surface of the cell wall (Fig. 8), and varies from 50 to 75 Å in thick-

ness. It is a differentiated surface of the cytoplasm, consisting of a lipid and a protein layer and functioning as an osmotic barrier. Active enzyme systems involved in protein synthesis are found on the surface of the cytoplasmic membrane.

The cell wall ranges from 100 to 800 Å in thickness and is laminated. It consists of three layers, the outermost being lipoprotein, the middle—lipopolysaccharide, and a rigid innermost layer of mucopolymers. The cell wall is present in true bacteria, and is absent in spirochaetes and myxobacteria. Bacteria are capable of retaining their shape due to the multilayered structure of the cell wall. The cell walls possess osmotic permeability.

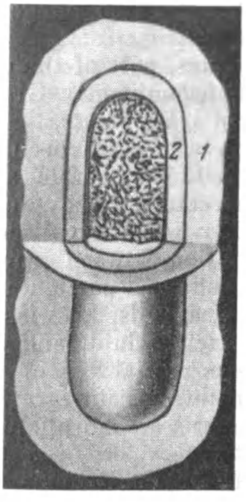


Fig. 8. Bacterial wall

1—cell wall; *2*—cytoplasmic membrane

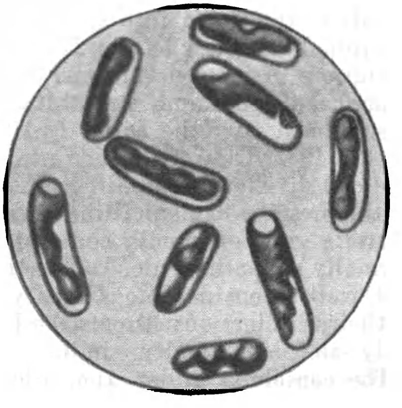


Fig. 9. Plasmolysis in bacteria

The process known as *plasmolysis* provides an illustration of the presence of a cell wall (Fig. 9). If bacteria are suspended in a hypertonic solution of salt or sugar, the cytoplasm becomes dehydrated, wrinkled and together with the cytoplasmic membrane pulls away from the cell wall. The cell wall maintains its shape and becomes markedly distinct.

With the help of special techniques, the cytoplasm can be destroyed, thus obtaining a substance consisting of a cell wall only. The cell walls of certain bacilli easily dissolve in lysozyme leaving naked protoplasts which possess many of the properties of bacterial cells.

The cell walls protect the bacteria from harmful environmental effects. Besides playing an active part in biochemical transformations, one of the functions of the cytoplasmic membrane is to regulate osmotic pressure.

Bacterial protoplasts. Bacteria deprived of the cell wall are known as protoplasts. They are spherical in shape, are capable of cell division, growth, respiration and sporulation and synthesize proteins, nucleic acids and enzymes.

Despite their resemblance to bacteria, protoplasts bear a number of essential differences. They are unstable structures very sensitive to the influence of osmotic pressure, mechanical action and aeration. They are unable to synthesize the component parts of the cell wall (diaminopimelic and muramuic acids), are resistant to phage infections, and are not capable of active motility.

If due to the action of lysozyme or other factors, part of the cell wall is dissolved, then the rod-shaped cells, predominantly Gram-negative, transform into spherical bodies called *spheroplasts.*

Protoplasts include the L-forms of bacteria which are peculiar variations of microorganisms. Morphologically, they resemble large spherical-shaped and thread-like plasmatic structures. They have been named after the Lister Institute where they were first discovered in 1935. They are formed when the coordination between growth and cell division is impaired. Their growth is enhanced particularly in the presence of penicillin. In contrast to typical cells, L-forms of bacteria grow on media containing serum or ascitic fluid, and are normally nonpathogenic. Due to their complete or partial loss of the cell walls, considerable fragility and a facilitated disintegration of their cellular constituents the L-forms of bacteria are morphologically and biologically similar to protoplasts.

The capsule. Under the influence of different environmental factors, some microbes are able to accumulate on their cell surface a thick slime layer surrounding the cell wall, known as a capsule. The capsular material consists of polysaccharides, glucoproteins or polypeptides and in some species—of proteins. It has a weak affinity to dyes and stains poorly (Fig. 10). The production of a capsule by microbes is considered as adaptation to environmental conditions. Pathogenic capsulated microbes are resistant to phagocytosis and to the effect of antibodies. A capsule is not a necessary part of the cell. With the aid of enzymes the slime layer can be digested without injury to the bacteria, although their infective capacity is lowered. The presence of a slime layer protects the capsulated microbes from desiccation.

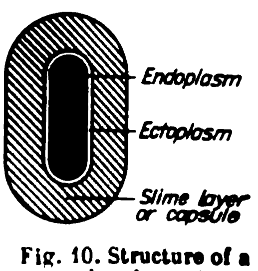


Fig. 10. Structure of a capsular bacterium

In some bacterial species the capsular material is associated with their type spe-

cificity which can be determined by the polysaccharide complex of the capsule.

There are some microbes which produce capsules only within the bodies of animals or humans (pneumococcus, anthrax bacillus, *Clostridium perfringens*). Other microbes produce capsules both within and without the body (bacteria of pneumonia, rhinoscleroma and ozaena).

The majority of microbes are able to produce capsules, particularly when cultivated in nutrient media of a high carbohydrate content. Saprophytic bacteria (*Leuconostoc*) are capable of producing a common capsule within which are contained a number of cells. These clumps of microorganisms held within a surrounding capsule are called *zoogleas.*

Special diagnostic techniques are employed to detect the capsule. In spite of the fact that capsular production is a species property, the bacterium can be deprived of it in natural as well as artificial conditions.

Flagella. Motile bacteria are subdivided into creeping and swimming bacteria. *Creeping bacteria* move slowly (creep) on a supporting surface as a result of wave-like contractions of their bodies, which cause periodic alterations in the shape of the cell. These bacteria include *Myxobacterium*, *Beggiatoa*, *Thiothrix*. *Swimming bacteria* move freely in a liquid medium. They possess flagella, thin hair-like cytoplasmic appendages measuring 0.02 to 0.05 μ in thickness and from 6 to 9 μ in length. In some spirilla they reach a length of 80 to 90 μ.

Investigations have confirmed that the flagella are made up of proteins the composition of which differs considerably from that of the bacterial cell proteins. With the aid of paper chromatography, it has been discovered that the flagellate material contains several amino acids: lysine, aspartic and glutamic acids, alanine, etc. It has been suggested that the flagella are attached to basal granules which are found in the outlying zones of the cytoplasm.

The flagella can be observed by dark-field illumination, by special methods involving treatment with mordants, adsorption of various substances and dyes on their surfaces, and by electron microscopy. The latter has made it possible to detect the spiral and screw-shaped structure of the flagella. The axial filament of the flagellum consists of two entwined hair-like processes enclosed in a sheath.

According to a pattern in the attachment of flagella motile microbes can be divided into 4 groups (Fig. 11): (1) *monotrichates*, bacteria having a single flagellum at one end of the cell (cholera vibrio, blue pus bacillus), (2) *amphitrichates*, bacteria with two polar flagella or with a tuft of flagella at both ends (*Spirillum volutans*), (3) *lophotrichates*, bacteria with a tuft of flagella at one end (blue-green milk bacillus, *Alcaligenes faecalis*), (4) *peritrichates*, bacteria having

flagella distributed over the whole surface of their bodies (colibacillum, salmonellae of enteric fever and paratyphoids A and B).

The above mentioned classification is provisional. While studying the flagella under an electron microscope, it was revealed that the flagellum in some monotrichates is not located at the end of the cell, but at the point of transition of the lateral surface to the pole. It has been established that bacteria which once were considered to be monotrichous possess a number of flagella. As to amphitrichates, their independent existence is a subject of controversy. It has been suggested that the amphitrichate cell is actually comprised of two

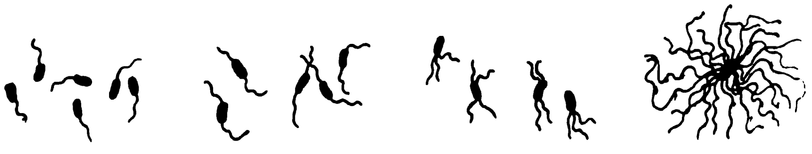


Fig 11. Bacterial flagella

1—monotrichates; *2*—amphitrichates; *3*—lophotrichates; *4*—peritrichates

cells which have been separated incompletely, having flagella at their distal ends.

Motility in bacteria with the help of flagella may be the result of periodic contractions along a spiral screw-shaped axis. It is quite possible that a thermokinetic process is basically involved in this motion. It has been suggested that the heat formed as a result of metabolism is given off to the environment through the flagella, while ATP serves as an energy source. The created difference in temperature causes a stream of water along the flagella, and the bacterium moves in an opposite direction. There are also other points of view on the mechanism of motility in bacteria.

The type of motility in bacteria depends on the number of flagella, age and properties of the culture, temperature, amount of chemical substances and on other factors. Monotrichates move with the greatest speed (60 μ per second). Peritrichates move at rates ranging from 25 to 30 μ per second. Certain species of motile microbes move at a rate of up to 200 μ per second.

Motility in bacteria can be observed by the hanging drop in wet conditions. The determination of motility in microbes is employed in laboratory practice as a means to identify cholera vibrios, dysentery, enteric fever, paratyphoid and other bacteria.

However, although the presence of flagella is a species characteristic, they are not always essential to life, since aflagellate forms of motile bacteria exist.

Several types of microbes possess *cilia* which are appendages considerably shorter and finer than flagella. There are from 100 to 250 cilia covering the cell

surface. They measure from 0.3 to 1μ in length and 0.01μ in width. It is considered that the cilia are not locomotor organs, but that they aid in attaching the microbial cell to the surface of certain substrates. It is possible that the cilia are involved in the nutrition of bacteria as they considerably increase the surface area of the bacterial cell.

Besides actively moving by means of flagella or by cell contraction, microbes are capable of *molecular*, passive or *brownian movement*. In 1827 R. Brown discovered that small particles suspended in a liquid or a gas are perpetually moving at random due to the thermal molecular motion of the surrounding medium. The motion of the particles in brownian movement can be explained by the mathematical laws of molecular statics. It is universal and does not depend on the nature of the dispersion medium or on the nature of the particles (whether living or nonliving matter). The rate of brownian movement depends on the size of the particles, on internal friction, viscosity of the medium and temperature. Particles from 5 to 10μ in size are subject to brownian movement, and no translational movement can be observed. If the particles are more than 50 to 100μ in size, no rotary movement will be noticed either.

Spores and sporulation. Endospores are small spherical or oval bodies formed within the cell. A spore is formed at a certain stage in the development of some microorganisms and this property was inherited in the process of evolution in the struggle for keeping the species intact. Some microorganisms, principally rod-shaped (bacilli and clostridia), are capable of sporulation. These include the causative agents of anthrax, tetanus, gas gangrene, botulism and also saprophytic species living in the soil, water and bodies of animals. Spore formation only rarely occurs in cocci (*Sarcina lutea*, *Sarcina ureae*), in spiral forms (*Desulfovibrio desulfuricans*), and in vibrios.

Sporulation occurs in the environment (in soil and on nutrient media), and is not observed in human or animal tissues.

The sporulation process occurs in four successive stages: (1) preparatory stage; (2) forespore stage; (3) stage of cell wall formation; and (4) maturation stage.

Under certain conditions, particularly unfavourable ones, structural changes take place inside the bacillus cell. The process is characterized by a thickening of the cytoplasm in a certain region and the formation of a forespore, which becomes surrounded by a thick poorly permeable multilayered wall. The rest of the cell gradually disappears. Instead of a vegetative cell, a mature spore, one-tenth the size of the parental cell, is produced. Sporulation is completed within 18 to 20 hours.

Spores are characterized by their high refractive index. If observed unstained under a microscope, they appear as glistening granules which stain poorly. Because of the presence of a thick multilayered wall having a laminated structure, a minimal free water content, and a high calcium and lipid content, the spores are capable of resisting unfavourable environmental conditions for many years. The spores of certain bacilli are capable of withstanding boiling and high concentrations of disinfectants. They are killed in an autoclave

exposed to saturated steam, at a temperature of 115-125°C, and also at a temperature of 150-170°C in a Pasteur hot-air oven.

When conditions become favourable, the spores germinate and transform again into vegetative cells. When germination occurs, the spores swell, enlarge in size, their water content increases, the rate of metabolic processes increases, and they stain easily with aniline dyes. From the wall at the pole, in the centre, or between the pole and centre an outgrowth begins to protrude which transforms into a rod. Usually germination takes place more quickly than sporulation (within 4 to 5 hours).

The sporulation process in bacilli is not one of multiplication, since most rod-shaped forms produce only one spore each.

In bacilli and clostridia, spores are located, Fig. 12, (1) *centrally*, in the centre of the cell (causative agent of anthrax); (2) *terminally*,

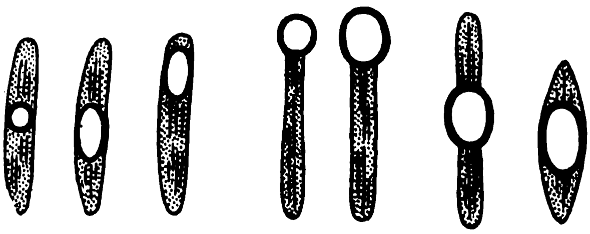


Fig. 12. Shapes and arrangement of spores in bacilli and clostridia

at the ends of the rod (causative agent of tetanus); (3) *subterminally*, towards the ends (causative agents of botulism, gas gangrene, etc.).

In some species of sporulating microorganisms, the spore diameter is greater than the width of the bacterial cell. If the spore is located subterminally, the microbes take on the form of a spindle (closter).

In tetanus clostridia the spore diameter is also greater than the width of the vegetative cell, but the spore is located terminally, and hence the drum-stick appearance.

This property of sporulation is important in characterizing and identifying spore-forming microbes, and also when selecting methods of decontaminating objects, housings, foodstuffs and other substances. The microbe may lose its ability to sporulate by frequent cultivation on fresh media or by subjecting it to high temperatures.

MORPHOLOGY AND STRUCTURE OF ACTINOMYCETES

Actinomycetes (Gr. *mykes*—fungus, *actis*—ray) are unicellular microorganisms which belong to the class *Schizomycetes*, the order *Actinomycetales*. The body of actinomycetes consists of *a mycelium*

which resembles a mass of branched, thin (0.2-1.2 μ in thickness), nonseptate filaments—hyphae (Fig. 13).

In some species the mycelium breaks up into poorly branching forms. In young cultures the cytoplasm in the cells of actinomycetes is homogenous, it refracts light to a certain extent, and contains separate chromatin grains. When the culture ages, vacuoles appear in the mycelial cells, and granules, droplets of fat and rod-shaped bodies also occur. The cell wall becomes fragile, breaks easily, and a partial lysis of the cells occurs. In actinomycetes, as in bacteria, differentiated cell nuclei have not been found, but the mycelial filaments contain chromatin granules. The actinomycetes multiply by means of germinating spores (Fig. 14) attached to sporophores (Fig. 15), and by means of fragmentation where they break up into hyphae.

The order *Actinomycetales* consists of 4 families: *Mycobacteriaceae*, *Actinomycetaceae*, *Streptomycetaceae*, *Actinoplanaceae*. The family *Mycobacteriaceae* includes the causative agents of tuberculosis, leprosy, and the family *Actinomycetaceae*, the causative agents of actinomycosis and acid-fast species nonpathogenic for man.

Among the actinomycetes of the family *Streptomycetaceae* are representatives which are capable of synthesizing antibiotic substances. These include producers of streptomycin, chloromycetin, chlortetracycline, oxytetracycline, neomycin, nystatin, etc. No species pathogenic for animals and man are present in the family *Actinoplanaceae*.

MORPHOLOGY AND STRUCTURE OF SPIROCHAETES

Genetically spirochaetes (Lat. *spira*—curve, Gr. *chaite*—cock, mane) are intermediate between bacteria and protozoa.

Spirochaetes differ from bacteria and fungi in structure, having a corkscrew spiral shape. Their size varies considerably (from 0.3 to 1.5 μ in width and from 7 to 500 μ in length). The body of the spirochaete consists of an axial filament and cytoplasm wound spirally around the filament. No distinct nucleus has been found. The spirochaetes do not possess the cell wall characteristic of bacteria, but electron microscopy has revealed that they have a thin cell wall (periplast) which encloses the cytoplasm. Spirochaetes do not produce spores, capsules, or flagella. Very delicate terminal filaments resembling flagella have been revealed in some species under the electron microscope.

In spite of the absence of flagella, spirochaetes are actively motile due to the distinct flexibility of their bodies. Spirochaetes have a rotating motion which is performed axially, a translational motion forwards and backwards, an undulating motion along the whole

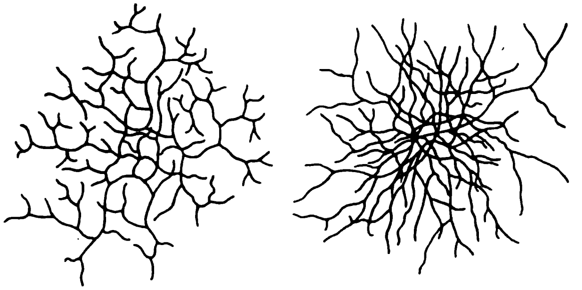


Fig. 13. General view of the mycelium in actinomycetes

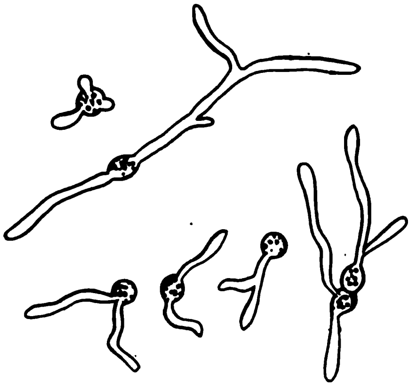


Fig. 14. Germination of spores in actinomycetes

Fig. 15. Structure of sporophores in actinomycetes

body of the microorganism, and a bending motion, when the body bends at a certain angle.

Some species stain blue, others blue-violet, and still others—pink with the Romanowsky-Giemsa stain. A good method of staining spirochaetes is by impregnation with silver.

Staining properties (reaction to stains) are used to differentiate between saprophytic and pathogenic representatives of spirochaetes.

Classification of spirochaetes. The order *Spirochaetales* consists of two families: *Spirochaetaceae* and *Treponemataceae.*

The former includes the saprophytes (*Spirochaeta*, *Saprospira*, *Cristispira*) representing large cells, 200-500 μ long, some have crypts (undulating crests), the ends are sharp or blunt (Fig. 16). They live

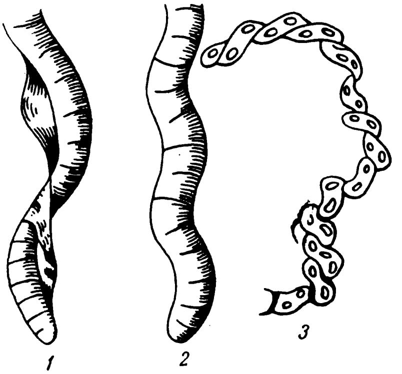


Fig. 16. Morphology of spirochaetes
1—Spirochaeta; 2—Saprospira; 3—Cristispira

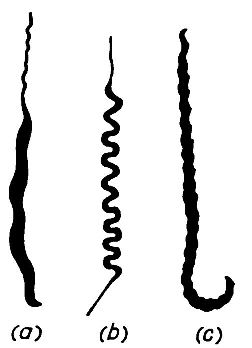


Fig. 17. *Borrelia* organism (*a*), *Treponema* (*b*), *Leptospira* (*c*)

on dead substrates, in foul waters, and in the intestines of cold-blooded animals. They stain blue with the Romanowsky-Giemsa stain.

Three genera belong to the family *Treponemataceae*: *Borrelia, Treponema*, *Leptospira* (Fig. 17).

The organisms of genus *Borrelia* (A. Borrel) differ from spirochaetes in that their cells have large, obtuse-angled, irregular spirals, the number of which varies from 3 to 10. Pathogenic for man are the causative agents of relapsing fever transmitted by lice (*Borrelia recurrentis*), and by ticks (*Borrelia persica*, etc.). These stain blue-violet with the Romanowsky-Giemsa stain.

The genus *Treponema* (Gr. *trepein*—turn, *nema*—thread) exhibits thin, flexible cells with 12-14 twists. They do not appear to have a visible axial filament or an axial crest when viewed under the microscope. The ends of treponemas are either tapered or rounded, some species have thin elongated threads on the poles. Granules of volu-

tin 40-90 mμ in diameter are found in the cytoplasm. The organisms stain pale-pink with the Romanowsky-Giemsa stain. A typical representative is the causative agent of syphilis—*Treponema pallidum*.

The genus *Leptospira* (Gr. *leptos*—thin) is characterized by its very thin cell structure. The leptospirae have a large number of closely wound coils in the shape of minute primary spirals. Leptospirae have rather complicated and active movements. The ends of the leptospira during movement spin rapidly bent at right angles to the main part of their body. During the stage of rest they look like hooks, while during rapid rotation they resemble button holes. Secondary spirals give the leptospirae the appearance of brackets or the letter S. The cytoplasm is weakly refractive. They stain pinkish with the Romanowsky-Giemsa stain. Some species which are pathogenic for animals and man cause icteric or nonicteric leptospirosis.

MORPHOLOGY AND STRUCTURE OF MYCOPLASMAS

The mycoplasmas belong to the class *Schizomycetes*, order *Mycoplasmatales*. These bacteria measure 100-150 mμ, sometimes 200-700mμ (a millimicron is equal to one-thousandth of a micron), are nonmotile and do not produce spores.

Mycoplasmas are the smallest microorganisms. They were first noticed by L. Pasteur when studying the causative agent of pleuropneumonia in cattle. However, at the time he was unable to isolate them in pure culture on standard nutrient media, or to see them under a light microscope. Because of this, these microorganisms were regarded as viruses. In 1898 E. Nocard and F. Roux established that the causative agent of pleuropneumonia can grow on complex nutrient media which do not contain cells from tissue cultures. W. Elford by means of special filters determined the size of the microbe to be within the range of 124-150 mμ. Thus in size, mycoplasmas appeared to be even smaller than some viruses.

Since they do not possess a true cell wall, mycoplasmas are characterized by a marked pleomorphism. They give rise to coccoid, granular, filamentous, cluster-like, ring-shaped, filterable forms, etc. Pleomorphism is observed in cultures and in the bodies of animals and man. No two forms are alike. The nuclear apparatus is diffuse. There are both pathogenic and nonpathogenic species. The most typical representative of the pathogenic species is the causative agent of pleuropneumonia in cattle (see p. 464).

At the present time more than 30 representatives of this order have been isolated, the most minute of all known bacteria. They are found in the soil, drainage waters, different substrates and in the bodies of animals and humans. Since mycoplasmas pass through many filters, and yet grow on media which do not contain live tissue

cells, they are considered to be microorganisms intermediate between bacteria and viruses. Chemically, mycoplasmas are closer to bacteria. They contain up to 4 per cent DNA and 8 per cent RNA.

MORPHOLOGY AND STRUCTURE OF RICKETTSIAE

According to their properties rickettsiae are intermediate between bacteria and viruses. They appear as pleomorphic microorganisms (Fig. 18).

Coccoid forms resemble very fine, homogenous or single-grain ovoids about 0.5 μ in diameter, quite often they occur as the diploforms.

Rod-shaped rickettsiae are short organisms from 1 to 1.5μ in diameter with granules on the ends, or long and usually curved thin rods from 3 to 4μ in length.

Filamentous forms are from 10 to 40 μ and more in length; sometimes they are curved and multigranular filaments.

Rickettsiae are nonmotile, do not produce spores and capsules and stain well by Morosov's method of silver impregnation, the Romanowsky-Giemsa stain and the Ziehl-Neelsen stain.

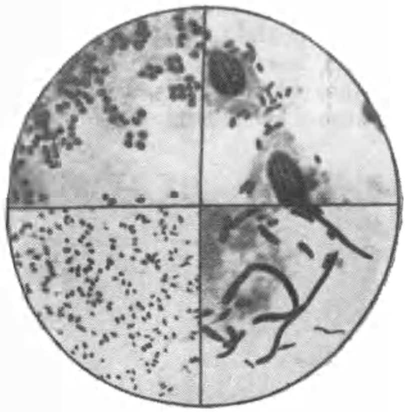


Fig. 18. Pleomorphism in rickettsiae

Electron microscopic and cytochemical studies of rickettsiae have revealed an outer marginal layer, a central body, and granular inclusions. The outer layer is from 5 to 10 mμ thick and functions as a cell wall; the intermediate region, 25 mμ in diameter, contains granules 5 mμ in size; and the central body is irregular in shape.

Rickettsiae multiply by division of the coccoid and rod-shaped forms which give rise to homogenous populations of the corresponding type, and also by the breaking down of the filamentous forms giving rise to coccoid and rod-shaped entities.

Pathogenic rickettsiae invade different species of animals and man. The diseases caused by rickettsiae are known as rickettsioses. A typical representative is *Rickettsia prowazekii* (the name was given in honour of the scientists, the American H. Ricketts and the Czech S. Prowazek), the causative agent of typhus fever.

Rickettsiae pertain to obligate parasites. They live and multiply only in the cells (in the cytoplasm and nucleus) of animals, humans and vectors.

The order *Rickettsiales* consists of 4 families: *Rickettsiaceae*, which has been characterized above; *Chlamydiaceae*—large (0.30-0.45 μ) microorganisms, the causative agents of ornithosis, psittacosis, trachoma, venereal lymphogranuloma, etc.; *Bartonellaceae*, parasites of human erythrocytes; *Anaplasmaceae*, parasites of animal erythrocytes.

MORPHOLOGY AND STRUCTURE OF VIRUSES

The name virus (Lat. *virus*—poison of animal origin) was given by L. Pasteur to many causative agents of infectious diseases, and by M. Beijerinck to the causative agent of tobacco mosaic disease. Later on, the term virus was used to designate those microorganisms which pass through candles, asbestos disks, and gradocol membranes (filterable viruses). Now it has been established that many microbes of a bacterial nature are capable of passing through such filters. That is why the term filterable virus is not employed today.

Fig. 19. Tobacco mosaic disease virus

Viruses do not have a cellular structure and are small in size, varying over a wide range from 10 to 350 mμ.

Morphology. Viruses may be classified in relation to their shape into several groups.

1. *Spherical form*. This includes the viruses of influenza (see Fig. 133), parotitis, Japanese encephalitis, fowl sarcoma, virus-like bodies of human tumours, etc. The size of viruses having a spherical shape varies within the range of 18 to 150 mμ.

2. *Rod-shaped form*. This includes the causative agents of tobacco mosaic disease (Fig. 19), potato blight, etc. They are 300 mμ in length and 15mμ in width.

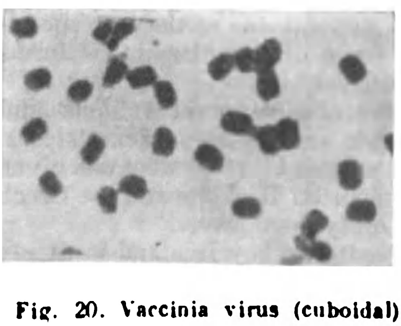


Fig. 20. Vaccinia virus (cuboidal)

3. *Cuboidal form*. This form pertains to viruses of vaccinia (Fig. 20), canary pox, molluscum contagiosum, etc. They are from 210 to 305 mμ in size.

4. *Spermatozoid form*. It is

characteristic for viruses of the lower plants (phages). Their size varies from 47-104 to 10-225 mμ.

The size of viruses can be determined by (1) filtration through colloid membranes, (2) centrifugation in high-speed centrifuges, and (3) electron microscopy (Table 2).

Table 2

Size of Viruses (in millimicrons), According to Different Methods

Virus	Methods of determination		
	Ultrafiltration	Ultracentrifugation	Electron microscopy of purified slides
Rabies (street)	100-150	—	110-120
Cowpox	125-175	170-180	227-305
Tobacco mosaic disease	15-25	—	15-300
Chickenpox	—	—	150-200
Dengue	20-30	—	—
Herpes	100-150	180-220	133-233
Parotitis	—	—	233±35
Measles	—	—	90-100
Influenza A2	80-120	70-116	123±1.18
Influenza B	—	97.3	123
Phages of some bacteria	—	—	heads 47-104 tails 10-225
Poliomyelitis	8-12	28	12-50
Japanese encephalitis	20-30	—	—
Yellow fever	17-27	—	50-55
Foot-and-mouth disease	8-12	17-20	20-32

A virus is composed of nucleic acid (DNA or RNA), and a protein wall containing enzyme systems with the help of which the virus penetrates into tissue cells. The cell wall or the special surface membrane has been named a capsid as it is composed of a certain amount of capsomers.

The nucleic acids are located inside the viral proteins. X-ray analysis of the viral structure has shown that the RNA of the tobacco mosaic virus is embedded deeply in the protein, and is not, as had been formerly presumed, the axis on which the protein disk-like subunits are strung. The nucleic and protein components are found in a strict dimensional relation. Some viruses are characterized by a complex structure, others—by a simple structure. The vaccinia virus contains protein, DNA, lipids, carbohydrates, riboflavin, biotin and copper. The tobacco mosaic virus is composed of protein and RNA, including 2.5 per cent of carbohydrates and 2.2 per cent of ash elements.

After treatment with a dilute solution of sodium hydroxide the virions (separate specimens of viruses) swell, and then rupture releasing their contents through the surface membrane which shows up as "shadows" on slides. In hypertonic solutions of saccharose

the volume of viral bodies decreases, while in hypotonic solutions it increases.

In a number of virus infections intracellular inclusions are formed. Many of them are well discerned under a light microscope, and are employed for laboratory diagnosis of rabies, smallpox and other diseases.

Viruses are obligate parasites. They live and multiply in the cells of live organisms (lower and higher plants, arthropods, wild and domestic animals, and man), but are also able to develop in homogenates of different tissues and organs.

Many species of viruses are pathogenic for man and cause diseases such as smallpox, rabies, certain tumours, influenza, measles, encephalitis, epidemic hepatitis, haemorrhagic fever, poliomyelitis, foot-and-mouth disease, etc. Viral diseases make up almost three-fourths of all human infectious diseases.

The nature of viruses. The problem of evolution of viruses and their nature is the subject of numerous investigations and theoretical discussions.

Some scientists consider viruses to be noncellular forms of a live parasitic system functionally closely bound with the host cell, but developing independently and genetically free from it. Others consider viruses to be cellular genetical factors capable of synthesizing the protein cell wall which protects them from the ill-effects of environmental factors and allows them to penetrate into cells. Certain workers do not agree that the viruses are live, and relate them to substances—transmissive nucleoproteins which have pathogenic properties and are constantly arising from cellular substance.

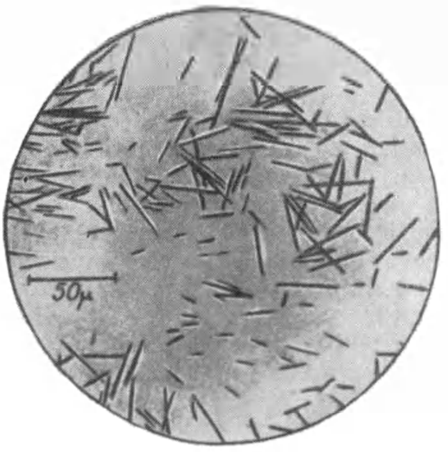


Fig. 21. Crystals of tobacco mosaic disease virus

In 1935 W. Stanley crystallized the virus of tobacco mosaic disease (Fig. 21). The crystals retained all the properties of tobacco mosaic disease. According to chemical structure, this virus is a nucleoprotein having a rod-shaped form and a molecular weight of 50,000,000. It consists of 94 per cent of protein and 6 per cent of nucleic acid.

In 1955 G. Fraenkel-Conrat and R. Williams resynthesized from separate biologically inactive components a typical tobacco mosaic virus which produced a characteristic disease in plants. The same authors isolated ribonucleic acid from protein.

An analogous result was obtained in 1963 by G. Cochrane and co-workers while studying noncellular extracts from leaves infected by the tobacco mosaic virus.

The resynthesis of viruses in laboratory conditions opens great perspectives in studying many problems of virology. It has been established that other plant viruses can occur in a crystallized state. In recent years it has been confirmed that nucleic acid retains the infective properties, while the cell wall does not take part in this process. Thus, for example, nucleic acids isolated by the phenol deproteinization method from viruses of influenza, foot-and-mouth disease, and tick-borne encephalitis were capable of infecting chick embryos, tissue cultures and susceptible laboratory animals. However, this extremely important problem calls for the accumulation of sufficient experimental data in order to affirm such a crucial conclusion of a general biological phenomenon.

The ability of certain viruses to form true crystals as observed in minerals and inorganic salts affirms the point of view of the nonliving nature of viruses. It is known that bacteria and rickettsiae do not form crystals.

Later, during a more profound investigation, it appeared that crystallization does not solve the problem of the nature of organisms. Crystallization depends on the mass and structure of particles forming the crystalline lattice. The mass of such bodies as bacteria, rickettsiae and large viruses is rather too great for them to be the structural units of crystals, while comparatively smaller viruses due to cohesive intramolecular forces and physicochemical structure of the specimens are able to crystallize.

At the present time a sufficient amount of data has been collected which confirms the scientific theory that life can occur not only in cellular, but also in noncellular forms of complex structures composed of nucleoprotein molecules. Viruses belong to these noncellular forms.

Classification of viruses. As yet there is no generally accepted and scientifically confirmed classification of viruses. Attempts have been made by some authors to classify viruses according to different characteristics. As a criterion for the taxonomy of viruses, their size, chemical composition and physical properties, amount of DNA and RNA, antigen structure, relation to chemical and physical effects, resistance to ether in particular, routes of transmission of viruses, tissue tropism, pathological and anatomical changes and clinical manifestations are taken into consideration.

In accordance with an international declaration, a temporary classification of viruses has been accepted in which all viruses are subdivided into a number of groups (see p. 35).

MORPHOLOGY AND STRUCTURE OF FUNGI

Fungi (Lat. *fungus*—mushroom) belong to the group of plant heterotrophic organisms which lack chlorophyll. The cells of fungi possess differentiated nuclei, and most of them multiply by sporulation. They differ considerably from bacteria. The phylum of true fungi (*Eumycophyta*) contains more than 80,000 species, and is subdivided into 4 classes.

1. **Phycomycetes**, fungi with a unicellular, nonseptate mycelium (500 species). The spores (endospores) are enclosed in special sporangia. Reproduction is sexual and asexual.

The genus *Mucor* or bread mould belongs to the class *Phycomycetes* (Fig. 22). It consists of a nonseptate mycelium in the shape of a much-branched cell, from which branch out the fruiting hyphae — *sporangiophores* with round dilations at the tips—*sporangia*. The latter are filled with endospores which provide a means of reproduction. *Mucor* mould may reproduce sexually too. It is widespread in nature, is often found on vegetables, moist surfaces of objects and in manure.

A typical representative of *Mucor* mould is *Mucor mucedo*.

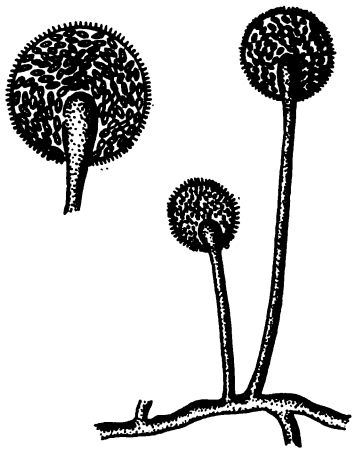


Fig. 22. *Mucor*

Pathogenic species of this mould may cause infections of the lungs and middle ear, and a general severe infectious process in humans.

2. **Ascomycetes** or sac fungi (35,000 species) have a multicellular mycelium. They reproduce sexually by means of ascospores (spores which develop in special spore cases, asci). The organisms reproduce asexually by means of conidia (exospores which bear the function of asexual reproduction in many fungi).

The genus *Aspergillus* belongs to the class *Ascomycetes*. The fungi have divided septate mycelium, and a unicellular conidiophore which terminates in a fan-like row of short sterigmata from which the spores are pinched off in chains—conidia (Gr. *konis*—dust).

Microscopic investigations have revealed that the fruiting part of the aspergillus (arrangement of endospores) resembles a jet of water from a watering can, and hence, the name "sprinkler" mould (Fig. 23).

A typical representative of aspergilla is *Aspergillus niger* which is widespread in nature. It is found on moist objects, on bread and jam. Certain species may cause aspergillosis of the lungs, ear and eye in humans or may infect the whole body.

The genus *Penicillium* belongs to the class *Ascomycetes*. The mycelium and conidiophore are multicellular, the fruiting body being in the shape of a brush. The conidiophore branches towards its upper part and terminates in sterigmata from which even-rowed chains of conidia are pinched off (Fig. 24). This genus of fungi is widespread in nature. It is found in fodder, milk products, on moist objects, old leather, in ink and jam. The type species is *Penicillium glaucum*. Certain species (*Penicillium notatum*. *Penicillium chrysogenum*, etc.) are used for producing penicillin which is widely em-

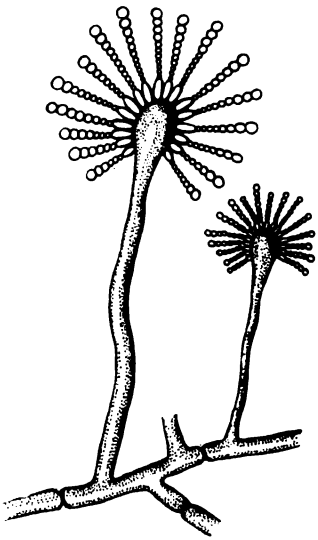


Fig. 23. *Aspergillus*

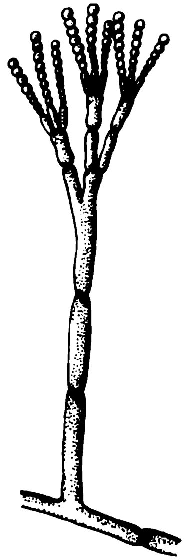


Fig. 24. *Penicillium*

ployed in treating many infectious diseases. Some species of this genus of fungi are pathogenic for humans. They cause infections of the skin, nails, ears, upper respiratory tract, lungs and other organs.

To the class *Ascomycetes*, the order *Protascales* (primary sac fungi) belong *the yeasts* which are large, oval, round and rod-shaped cells (Fig. 25). Yeast cells have a double-cell wall and a well defined nucleus. The cytoplasm is homogenous, sometimes of a fine granular structure. It contains inclusions (glycogen, volutin, lipid) and vacuoles, and also filamentous bodies—chondriosomes, which are involved in synthetic processes in the cell. Yeasts multiply by budding, fission, sporulation. Some species of yeasts reproduce sexually. Daughter cells produced by budding from the parent cell transform into independent individuals.

True yeasts are capable of reproducing by sporulation. When there is a lack of nutrition, 2, 4, 8 or 16 endospores are formed inside the cells of some species of yeast. The yeast cell forming the ascospores is called the ascus (sac), while sporulating yeasts are known as *Ascomycetes*.

Many species and varieties of this genus of yeasts are capable of fermenting different carbohydrates. They are widely used in brewing beer, in wine making and baking bread. Typical representatives of these yeasts are *Saccharomyces cerevisiae*, and *Saccharomyces ellipsoides*.

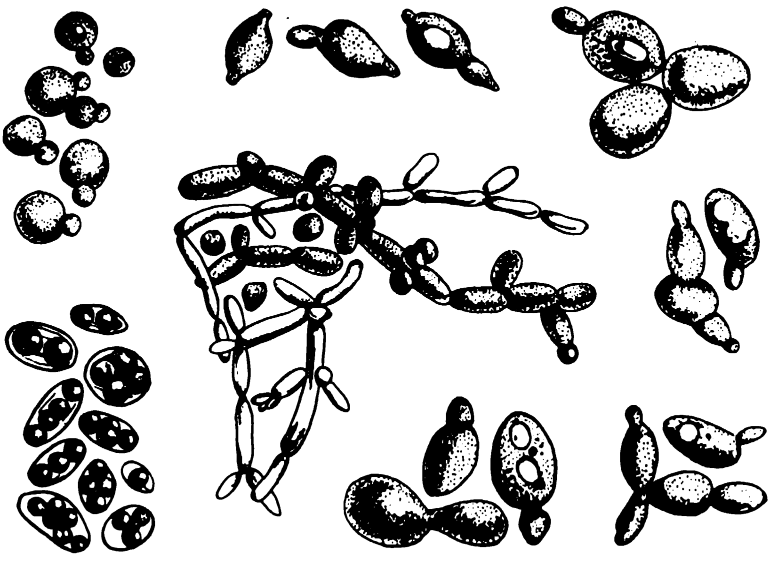


Fig. 25. Yeasts

Included among the asporogenic yeasts (family *Saccharomycetaceae*, subfamily *Non-ascoendomycetaceae*) are the species pathogenic for man from the genus *Candida* (Fig. 136) which cause grave diseases known as candidiases. They occur as a result of the growth inhibition of the normal microflora by antibiotics used for treating a number of infectious diseases and inflammatory processes, and also for treating grave diseases when the natural defense mechanisms of the body are weakened.

3. **Basidiomycetes**, fungi with a multicellular mycelium. These organisms predominantly reproduce sexually by basidiospores (basidia—reproductive organs in which a certain number of spores develop, usually 4). The majority of them live on decaying humus and vegetable matter. Certain species are tree parasites. Two hundred species of mushrooms are edible. The fruiting bodies which are commonly known as mushrooms are used as food. Twenty five species of mushrooms are poisonous.

Smut fungi invade grain crops causing a disease known as smut. Rust fungi affect sunflowers, and other plants, producing orange-coloured spots on infected plants.

4. **Imperfect fungi** (*Fungi imperfecti*) are a rather large group of fungi consisting of a multicellular mycelium without either the asco- or basidio-sporangiophore, but only with conidia. Reproduction is asexual, sexual reproduction being unknown. To this class belong the orders *Hyphomycetales*, *Melanconiales*, and *Sphaeropsidales*.

Among the hyphomycetes which may be of interest to physicians are: *Fusarium graminearum* causing intoxication in humans ("drunken bread"), and *Fusarium sporotrichioidales* causing intoxication in man and domestic animals who have eaten the grain crops which had remained in the fields during the winter.

Pathogenic species of imperfect fungi are causative agents of dermatomycoses: favus (*Achorion schoenleini*), trichophytosis (*Trichophyton violaceum*), microsporosis (*Microsporum lanosum*), epidermophytosis (*Epidermophyton inguinale*).

MORPHOLOGY AND STRUCTURE OF PROTOZOA

Protozoa (Gr. *protos*—first, *zoon*—animal) are unicellular animal organisms more highly organized than bacteria. They have a cytoplasm, a differentiated nucleus, a cell wall which differs in optical properties, and primitive organelles.

Protozoa reproduce by simple and multicellular division, sexually, and also by a more complicated process—sexually and asexually (malarial plasmodium). Amoebae, lamblias and balantidia can produce cysts which are more resistant forms for survival. Representatives of certain species have two or more nuclei. The phylum *Protozoa* can be subdivided into 5 classes:

Class 1 — **flagellates** (*Mastigophora or Flagellata*). Movement is effected with the help of one or several long flagella. They are oval, elongate or spherical in shape. Reproduction in parasites is generally by longitudinal fission, and more rarely sexual reproduction occurs. Type representatives are trypanosomes, leishmaniae and lamblias.

Class 2 —*Sarcodina or Rhizopoda*. These organisms move with the help of pseudopodia or cytoplasmic processes. The body of the microorganism easily changes its morphology. Reproduction is by simple cell division. The production of a cyst is one of the stages in the life cycle. Among the pathogenic species for man is *Entamoeba histolytica*.

Class 3 —*Sporozoa*. The members of this class have no special locomotor organs. Reproduce sexually and asexually. Plasmodia, causative agents of malaria in humans, animals and fowl, pertain to this class.

Class 4 —*Cnidosporidia*. These include the slime spore parasites mainly from fish. The microorganisms of the order *Microsporidia* predominantly infect insects.

Class 5 —*Infusoria*. These organisms move by means of cilia. The body shape is constant. The protozoa of this class have oral and anal openings. *Balantidium coli* is a species pathogenic for man.

A more detailed description and characteristic of protozoa is given in the biology course. The main information on pathogenic species is given in the section on special microbiology.

BASIC METHODS FOR INVESTIGATION OF THE MORPHOLOGY OF MICROORGANISMS

The shape, size and structure of bacteria are studied with the aid of *optical microscopes*, the construction of which is described in the physics textbooks and laboratory manuals. In ordinary modern microscopes employing the immersion system, the resolving power in visible light is within the range of 0.15 to 0.20 μ, which limits the possibility of studying the more minute structural parts of the bacterial cells and of viruses.

The use of photography in ultraviolet light has increased the resolving power of the microscope, and has permitted the study of minute-sized microorganisms and their structure. Especially good results have been obtained with microphotography.

For a more profound study of the fine structure of the bacterial cells and of viruses, microscopic investigations are carried out with the help of *the electron microscope* which gives a magnification up to 100,000 times. The electron microscope has a greater resolving power, a greater focal length and the penetrating power is of a low order. Since the thickness of the cell of most bacteria is smaller than the focal length of the electron microscope, it is impossible to reveal the positions of various structures without the use of additional methods. This defect has been overcome by using the shadow-casting method and stereoscopic microphotography. Thus the results obtained by using the light microscope are supplemented by electron microscopy and conversely, the light microscope can verify the results obtained by the electron microscope.

At the present time *phase contrast microscopy* is employed, which lessens the diffraction range and permits the investigation of the structural parts of the cell with a high degree of accuracy. By this method it is possible to obtain contrasting images of unstained living cells, which permits the study of bacteria in the process of growth and multiplication. For phase contrast microscopy the optical industry produces special accessories for conventional microscopes.

In order to eliminate weakly contrasting images in phase contrast microscopy *the anoptral method of microscopy* is employed. The latter has the advantage of giving greater resolving power and focal length and improving the stereoscopic effect. The halo of light around the object under investigation is eliminated.

In some cases *luminescent microscopy* is employed. With the aid of this method not only can the structure of the cell be revealed, but its functional state can be determined, and one can distinguish between live and dead cells.

The size of the bacterial cells is determined by means of *an ocular micrometer* or by microphotography.

The flagella in live bacteria can be examined by *dark-field microscopy* under a strong source of illumination and a comparatively

small magnification. For this purpose the microorganisms are treated with electrolytes and colloidal substances (methylcellulose, gum arabic, gelatin) which aid in increasing the thickness of the flagella up to the visual range.

For studying the processes of growth, multiplication and sporulation *microfilming* or *series microphotography* is employed.

Methods of investigation employing stained specimens are of great importance in microbiological practice. Basic, neutral, and acid organic dyes are used for this purpose. Little is known about the mechanism of staining, although it is known that the binding of the component parts of the cell with dyes involves physicochemical reactions. Some dyes differentially stain the component parts of the microbes. This is known as metachromasia, and is observed when staining diphtheria bacilli and other microorganisms. There are simple and differential staining techniques. In *simple staining* of bacteria a single dye (fuchsin, methylene blue, etc.) is used. In *differential staining* several dyes are employed. These include: Gram, Ziehl-Neelsen, Neisseria and Romanowsky-Giemsa stains, etc.

The Gram stain known since 1884 has not lost its practical significance. All bacteria stained by the Gram method can be subdivided according to colour into Gram-positive and Gram-negative. Some bacteria occupy an intermediate position, and are known as Gram-variable organisms. Flame-fixed smears are stained first by gentian violet (crystal violet, methyl violet), and then treated with Lugoll's iodine solution. As a result compounds are formed in the cytoplasm of the bacterial cell, which are retained by some bacterial species during decoloration with alcohol. The smears are washed with water and counterstained with an aqueous solution of fuchsin. Those bacteria which firmly retain the gentian violet colour, and which cannot be decolorized by alcohol, stain violet and are known as Gram-positive (staphylococci, see Fig. 117,*1*, streptococci, pneumococci, and the organisms responsible for diphtheria, tuberculosis, anthrax, tetanus, gas gangrene, etc.). Those bacteria which are decolorized by alcohol, and stain red are known as Gram-negative [gonococci, meningococci, brucella (see Fig. 117,*1*), colibacilli, salmonellae, cholera vibrio, causative agents of plague, tularaemia, etc.]. When using the Gram stain, it is necessary to avoid thick smears, to wash thoroughly with an organic solution (alcohol), and to take into account the age of the bacteria. Very young or old specimens of Gram-positive bacteria frequently stain Gram-negative. Under the influence of antibacterial preparations, Gram-positive microbes can become Gram-negative.

There is a certain relationship between Gram stain and certain bacterial properties. Gram-negative microorganisms plasmolyse easier, they have a higher isoelectric point, are less sensitive to the action of halides, triphenylmethane dyes and to the bactericidal substances in blood serum. The principle of the mechanism of the

Gram stain involves physicochemical processes related to compounds containing phosphorus and nucleic acids. Gram-positive microbes have stronger acidic properties, adsorb most of the main dye (gentian violet), and when treated with a mordant retain the stain better than do the Gram-negative organisms. The cell wall of Gram-negative bacteria is thinner. It contains 10 times as much lipids and is chemically more complex than the cell wall of Gram-positive bacteria. The cell wall of Gram-positive bacteria makes up about 20 per cent of the dry weight of the bacterial mass, including some amino acids as diaminopimelic acid, hexosamines, and 1-3 per cent lipids.

The Ziehl-Neelsen stain is employed for differentiating acid-fast bacteria [bacilli of tuberculosis, leprosy (see Fig. 117,*4-5*), actinomycetes] which stain with difficulty. The smears are usually heat-fixed by passing through a flame, and then are stained intensively with basic phenol fuchsin. After this they are treated with a 3-5 per cent sulphuric acid solution, washed with water and counterstained with methylene blue. Acid-fast bacteria retain the red stain, while all other bacteria are stained blue. Acid-fastness in bacteria is related to the presence of a large amount of lipids, waxes and oxyacids.

To determine the shape and size of bacteria or the presence of a capsule, *negative staining* is employed. In this method the dye particles (Indian-ink, water-soluble nigrosin, Congo red, collargol) reach the outer margin of the slime layer, and thus the margin of the cytoplasmic membrane can be distinguished due to refraction. The distance between the two margins is equal to the thickness of the slime layer and cell wall (see Fig. 117,*9*).

Intravital staining can be performed by using a weak solution of basic dyes which are not toxic (methylene blue, cresol blue, Nile blue, neutral red). This method is used for studying volutin granules which stain more quickly and more intensely than the cytoplasm, and also for differentiating live and dead cells.

Microchemical methods are used to determine the solubility of lipids, volutin, cellulose, sulphur, the identification of vacuoles of cell sap and lipid inclusions, and the nature of carbohydrate and protein fractions. Bacteria can be studied by microdissection methods, with the aid of a micromanipulator or selector, ultracentrifugation, X-ray irradiation, or by a stream of electrons with certain characteristics, by means of filtration through bacterial filters, electrophoresis, infraspectrophotometry and other more complex and sensitive investigations, in particular ultrathin sections of bacterial cells, employing cytochemical methods and radioactive isotopes.

In the corresponding sections of the textbook information on the methods of investigation will be supplemented, although they cannot substitute for a special laboratory manual in which a detailed description of the modern methods of studying bacteria, actinomycetes, rickettsiae, viruses, fungi, and protozoa is given.

PHYSIOLOGY OF MICROORGANISMS

The study of the physiology of microbes is of great theoretical as well as practical significance. Owing to the rapid development of genetics, biophysics, biochemistry and electron microscopy, it has been possible to carry out investigations on the physiological processes in bacteria at the molecular level. Modern methods of studying microbes are carried out in relation to morphological, physicochemical, and physiological properties. A thorough analysis of the data concerning the morphology and physiology of microorganisms has revealed that they are structurally and biochemically complex organisms (Fig. 26). Due to this they may rapidly adapt themselves to different environmental conditions, because of their ability to form adaptive (induced) enzymes produced by the influence of new substrates of the surrounding environment. Consequently the production of adaptive enzymes causes a change in the character of metabolism and biological functions of the new variants.

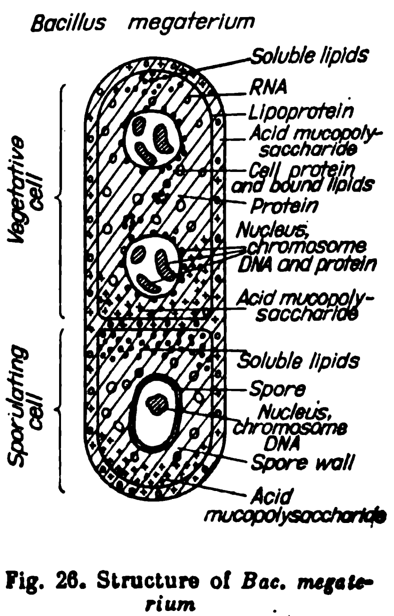


Fig. 26. Structure of *Bac. megaterium*

CHEMICAL COMPOSITION OF MICROORGANISMS

The bacterial cell contains the main chemical elements, organogenes—nitrogen, carbon, oxygen and hydrogen. The percentages (dry matter) of nitrogen and carbon are 8-15 per cent and 45-55 per cent, respectively.

From the various elements and their compounds microorganisms synthesize proteins, nucleoproteins, carbohydrates, lipids, lipoglycosides, lipoglucoprotein complexes, nucleic acids, enzymes, vitamins, etc.

Water. The water content in the cytoplasm of most species of bacteria varies from 75 per cent (colibacilli) to 85 per cent (diphtheria bacilli, tubercle bacilli, cholera vibrio). Water is the main component of the cell, and is found free and bound with other component substances. *Bound water* is a structural element of the cytoplasm, and cannot be a solvent. *Free water* serves as a dispersion medium for colloids, and as a solvent for crystalline substances, as a source of hydrogen and hydroxyl ions, and is involved in chemical reactions. For example, the hydrolytic processes of the breakdown of proteins, carbohydrates and lipids take place as a result of the binding of water. Water plays an important part in the processes of respiration.

Mineral substances. Inorganic substances (phosphorus, sulphur, sodium, magnesium, potassium, calcium, iron, silicon, chlorine, etc.) and trace elements (molybdenum, cobalt, boron, manganese, zinc, copper, etc.) are also found in the bacterial cell. The total amount of mineral substances in bacteria grown on standard nutrient media varies from 2 to 14 per cent of the dry matter of the microbial mass.

Dry matter. The organic part of the dry matter of bacteria consists of proteins, nucleic acids, carbohydrates, lipids, and other compounds.

Proteins. More than 50-80 per cent of the dry matter of the bacterial cell is made up of proteins found in the cytoplasm, nucleus, cytoplasmic membrane and other cell structures.

Proteins are composed of certain nucleoproteins, the prosthetic group of which is made up of nucleic acids. Lipoproteins pertain to the second component part of proteins. Their prosthetic group is made up of fats (lipids) or fat-like substances (lipoids). Lipoproteins are found within the cell as inclusions having a semisolid consistency, including mitochondria. On the surface of the cytoplasm lipoproteins form a membrane which regulates the substances entering the bacterial cell. Enzymes which play an important part in the life processes of microorganisms are proteins which have prosthetic groups (active groups). The protein part of the enzyme (apoenzyme) has a specific function, and the prosthetic group carries out a chemical reaction. In some cases the prosthetic groups are not bound firmly to the protein and easily separate from it, while others can bind themselves to different proteins. These freely existing non-protein catalysts involved in biochemical transformations are known as coenzymes. Another group of enzymes contain haemin compounds as the active group. Enzymes concerned with oxidation belong to this group.

Nucleic acids. The amount of nucleic acids in the bacterial cell depends on the bacterial species and the nutrient medium, and varies within 10-30 per cent of the dry matter. Nucleic acids are normally bound to proteins and complex radicals of the cell structures of bacteria. Ribonucleic acid takes part in the synthesis of proteins, and desoxyribonucleic acid determines hereditary properties. DNA is composed of adenine, guanine, cytosine, thymine, phosphoric acid, and desoxyribose; RNA is composed of adenine, guanine, cytosine, uracil, phosphoric acid, and ribose. Thus the difference between these two nucleic acids is that DNA contains the nitrogenous base, thymine, and desoxyribose, while RNA contains uracil and ribose.

It is important to note that for every species of bacteria there is a definite ratio of paired bases $\frac{\text{guanine}+\text{cytosine}}{\text{adenine}+\text{thymine}}$ (see p. 32).

This qualitative and quantitative variation of proteins, their complexes and amino acids gives the microorganisms their type specificity.

Carbohydrates. Carbohydrates and polyatomic alcohols comprise 12-18 per cent of the dry matter in the bacterial cell. These include (1) polyatomic alcohols; (2) oligosides; (3) polyosides; (4) neutral oligopolyosides containing N-acetylamino groups; (5) acid polyosides; (6) oligo- and polyosides containing sialic acid. The main part of the carbohydrates is a polysaccharide complex, free from or bound with proteins and lipids, found in the cell wall and slime layer. The cytoplasm of many bacteria has a comparatively large amount of inclusions chemically resembling glycogen or starch.

Polysaccharides. The polysaccharides of the capsules of type II, III, VIII pneumococcus pertain to the group of compounds which do not contain nitrogen. They are polymers of aldobionic acid, and during complete hydrolysis break down into glucose and glucuronic acid. The polysaccharides of other microbes include dextrins, levans (fructosans), and cellulose.

Some microbes have hexosamines which on hydrolysis break down into monosaccharides, aminosaccharides and amino acids (type I, IV, XIV pneumococcus, bacilli of diphtheria, tuberculosis, etc.). During acid hydrolysis of polysaccharides, galactose, glucose, levulose and other monosaccharides are released.

The type specificity of microbes depends on the polysaccharide fractions. This is of great significance in laboratory diagnosis, in the preparation of vaccines, and medicinal and diagnostic sera.

Lipids. In those bacteria which do not store fat in the form of inclusions, lipids make up almost 10 per cent of the dry matter (in diphtheria bacilli—5 per cent). In those bacteria which store fat as special inclusions, the amount of lipids attains 40 per cent. Bac-

terial lipids are made up of free fatty acids (26-28 per cent), neutral fats, waxes and phospholipids.

The lipids of enteric fever bacteria are almost exclusively free fatty acids (palmitic, stearic, oleic and also lauric, myristic, tetracosanic, butyric, caproic, etc.)

Tuberculostearic, oxystearic, palmitostearic, phthienic and phthioic acids have been extracted from tubercle bacilli and diphtheric acid, from diphtheria bacilli.

Neutral lipids of bacteria consist of fatty acid ethers and carbohydrates. The amount of glycerin and stearin varies in the range of 2.5-12.5 per cent of the total amount of lipids.

The tuberculosis bacilli contain 12-15 per cent of the bound lipids composed of carbohydrates and mycolic acid. They contain a large amount of wax which during saponification releases up to 84 per cent of high-molecular fatty oxy-acids. The latter possess a characteristically high resistance to the action of mineral acids, alcohols and alkalies.

The amount of phosphatides in bacterial lipids varies from 0.4 to 6.5 per cent of dry matter. Fatty acids, polysaccharides and a mixture of glycerophosphoric acid and choline are obtained on hydrolysis of phosphatides.

The chemical composition of the microbial cell depends on the substances contained in the nutrient medium, the nature of metabolism and environmental conditions.

The chemical composition of actinomycetes and spirochaetes is fundamentally similar to that of bacteria. The difference lies in the quantitative proportions of separate elements and their complex structures.

Chemical composition of rickettsiae. Rickettsiae contain proteins, nucleoproteins, carbohydrates and lipids, and have enzyme systems. In the large rickettsiae catalase and phosphatase have been found. There is a toxic substance made up of a protein complex in the body of rickettsiae. Rickettsiae contain a large amount of DNA. Different species of rickettsiae have different ratios of $\frac{\text{guanine}+\text{cytosine}}{\text{adenine}+\text{thymine}}$. In *Rickettsia prowazekii* this ratio is equal to 1.25, in *Rickettsia burnetii*—2.08.

Chemical composition of viruses. The elementary composition of viruses does not differ from that of plant, animal and bacterial proteins. The chemical composition of viral complexes differs from that of bacteria. The main component of viruses is the nucleoprotein.

All viruses can be subdivided into two groups: (1) viruses containing DNA (adenoviruses, poxviruses, phages, etc.), and (2) viruses containing RNA (viruses of plants, viruses of influenza, encephalitides, haemorrhagic fevers, poliomyelitis, Coxsackie and ECHO viruses, etc.). Comparative data concerning the chemical composition of some viruses are given in Table 3.

Table 3

General Chemical Composition of Certain Viruses

Virus	Protein	Nucleic acid		Carbo-hydrates	Lipids
		DNA	RNA		
Vaccinia	62.0	0.8	—	4.0	33.2
Encephalomyelitis	49.1	6.8	—	4.0	40.1
Influenza type A	65.0	—	1.0	10.6	23.4
Phage of *E. coli*	52.4	40.0	—	7.6	are found

The proteins of viruses are built up of 16-20 basic amino acids. Dicarboxylic oxy-acids predominate in the viral protein. The amino acids are arranged in a particular sequence according to their C- and N-amino groups, the type and amount of which are characteristic for each virus.

The structure of nucleic acids was deciphered by the investigations of J. Watson and F. Crick, who established that DNA is composed of two polynucleotide chains spirally wound and joined by hydrogen bonds between the 6-amino and 6-oxy-groups.

Besides proteins and nucleic acids, the virus has been shown to contain carbohydrates and lipids, which play an important part in the defense mechanisms of viruses.

The influenza virus contains nucleoproteins, lipids, carbohydrates and an enzyme haemagglutinin (neuraminidase). Certain viruses contain mucinase (viruses of parotitis, swine influenza), adenosinphosphatase (virus of fowl myeloblastosis), cytochrome reductase (virus of meningopneumonia), and haemolysine (viruses of parotitis, false fowl plague, parainfluenza viruses).

Many viruses are composed only of nucleoproteins (plant viruses, phages, viruses which cause poliomyelitis and rabbit papilloma, etc.).

The colibacillus phage contains 17 amino acids. In some types of phages of these bacteria desoxyribonucleic acid makes up almost 40 per cent of the weight. The protein and desoxyribonucleic acid of phages give them their species specificity. The enzyme of the lysozyme-type phage promotes the penetration of DNA of the phage into the cytoplasm of the affected bacteria.

PHYSICOCHEMICAL PROPERTIES OF MICROORGANISMS

The average *specific weight* of bacteria is 1.055. *The viscosity* of the bacterial cytoplasm is greater than that of water and may be from 3 to 800 times as dense as water. All kinds of physical and chemical injury cause first of all a reversible and then an irreversi-

ble coagulation of the cytoplasm, and an increase in the viscosity and staining properties of the bacterial body.

The bacteria possess *elasticity*. Their inner contents occur as a colloidal sol while the surface layers are in a gel state.

The bacterial wall determines the various shapes of the bacteria. The cytoplasmic membrane is a biologically semipermeable membrane through which substances enter the cell and metabolic products are excreted by means of diffusion and osmosis.

The intracellular osmotic pressure in bacteria is half that of the cells of higher animals. In the old cells of Gram-negative bacteria it is 2-3 atmospheres. In young growing cultures of colibacilli and staphylococci, the intracellular osmotic pressure reaches 15-20 atmospheres.

The permeability of the bacterial cell is greater than that of the animal cells, thus their isotonic solutions are different. Some bacteria require a 0.3 per cent salt solution, while others (inhabitants of the sea and salt lakes) require a 3-25 per cent solution. Most pathogenic bacteria develop in nutrient media containing 0.5 per cent salt. There are bacteria which grow on media containing only water. These data are put into practice for preparing nutrient media to which definite amounts of sodium chloride should be added to obtain isotonicity.

In conditions of hypertonic salt or sugar solutions, the cytoplasm becomes dehydrated and the bacteria perish. This principle is used in the methods of conservation of foodstuffs (salting of meat, fish, vegetables, preparation of jams, candied fruits, etc.).

In weak neutral solutions of sodium chloride the majority of bacteria have a negative charge (spirochaetes have a positive electric charge), and bacteria in the free suspended state in an aquatic neutral medium move towards the anode under the influence of an electrical current. Thus bacteria have a negative electrokinetic potential. Suspensions of bacteria are electronegative colloids and can be precipitated by many nonspecific agents (basic dyes), and also by acids at the isoelectric point with an electrokinetic potential equal to zero.

NUTRITION OF MICROORGANISMS

A constant exchange of compounds with the surrounding environment is inherent in all organisms. To carry out the processes of nutrition and reproduction certain conditions are necessary: the presence of food material from which microbes synthesize the component parts of their cell, and, by oxidation of different substances, receive the required energy.

Bacteria can be subdivided into autotrophic and heterotrophic according to their type of nutrition.

Autotrophic (Gr. *autos*—self, *trophe*—nutrition) chemosynthetic and photosynthetic microorganisms are able to produce organic substances from inorganic compounds. They do not require organic carbon compounds, and synthesize the component parts of their cell by absorbing carbon dioxide, water and simple nitrogen compounds (ammonia and its salts, the salts of nitric acid). Nitrifying bacteria and many sulphur bacteria belong to the autotrophic microbes. They synthesize complex substances at the expense of the energy which they receive on oxidation of ammonia to nitrites (*Nitrosomonas*) and nitrates (*Nitrobacter*), and oxidation of sulphur, sulphides, thiosulphates to sulphuric acid (*Thiobacillus thiooxidans*).

Some species of microorganisms—anaerobic purple and green sulphur bacteria (*Thiorhodaceae*, *Chlorobacteriaceae*) contain chlorophyll, and utilize radiant energy for photosynthesis.

While studying autotrophic bacteria, it was established that during the process of synthesis of all the cellular organic substances, they utilize carbon dioxide as the sole source of carbon and are unable to absorb more complex carbon compounds. For this reason such organisms cannot be pathogenic for man and animals.

Heterotrophic (Gr. *heteros*—another) bacteria require organic carbon (carbohydrates, keto-, amino-, oxy-, and fatty acids), various nitrogen compounds (nitrates, ammonia), inorganic substances, trace elements and vitamins. Heterotrophic bacteria can be subdivided into saprophytes and parasites.

A. *Saprophytes* (Gr. *sapros*—decaying, *phyton*—plant) live at the expense of organic substances found in the surrounding environment. These include most species of bacteria inhabiting our planet. They are also called metatrophs.

B. *Parasites* (living on or in another body, and feeding at its expense). This group makes up a comparatively small amount of species of microbes which in the process of evolution have adapted themselves to a parasitic mode of life. Some scientists call them *paratrophs*, since they feed at the expense of organic compounds of animals and man. However, this kind of subdivision of heterotrophic microbes into saprophytes and parasites is not absolute, since such a distinct line between these subgroups cannot be established (see "Types of Symbiosis").

Certain species of microbes pathogenic for man can exist in the environment as saprophytes, and vice versa, some saprophytes under unfavourable conditions can cause different diseases in humans and animals.

At present it has been established that some microbes which earlier were considered to be typical heterotrophs grow well on synthetic media containing ammonium sulphate supplemented by vitamins. Many pathogenic microorganisms cultivated on media containing blood, ascitic fluid, serum, etc., can be grown on synthetic media.

The majority of bacteria develop only on complex media containing peptone (a product of enzymatic breakdown of meat and other protein substrates), meat extract and products of a similar biological origin, which contain all the organogenes in the form of highly molecular compounds necessary for the nutrition of microbes.

Nitrogen and its compounds are of great importance in the nutrition of microbes. According to the character of nitrogen nutrition B. Knight and J. Porter have subdivided microorganisms into a number of groups: (1) those fixing atmospheric nitrogen; (2) those absorbing mineral forms of nitrogen (ammonium sulphate); (3) those assimilating ammonium salts, nitrates or nitrites in the presence of amino acids or purines; (4) those growing in the presence of separate amino acids or their mixtures; (5) those cultivated in protein nutrient media.

The sources of carbon for microbes may be different carbohydrates, polyatomic alcohols, organic acids and their salts.

B. Knight has divided bacteria into 4 groups according to their ability to synthesize complex compounds.

1. Bacteria obtaining carbon from carbon dioxide, and nitrogen from inorganic compounds. These include autotrophs capable of photosynthesis. Such organisms utilize radiant energy (light). Autotrophs capable of chemosynthesis obtain energy by the simple processes of oxidation of inorganic compounds (nitrifying bacteria, sulphur bacteria, some iron bacteria).

2. Bacteria deriving carbon and obtaining energy from organic carbon compounds and nitrogen from its inorganic compounds (the majority of saprophytes).

3. Bacteria obtaining carbon and energy from organic carbon compounds and nitrogen from amino acids (colibacilli and other commensals).

4. Bacteria absorbing carbon and obtaining energy from organic compounds, and nitrogen from a complex of many amino acids, requiring one or more vitamins (pathogenic bacteria).

The main difference between heterotrophic and autotrophic organisms is that they require organic compounds containing an asymmetric carbon atom. However, recently it has been proved that separate species of heterotrophic bacteria, protozoa, yeasts and also animals absorb carbon dioxide and ammonia, synthesizing complex carbohydrates and amino acids from them.

These data support the theory stated in 1921 by A. Lebedev, that there are no absolutely heterotrophic microorganisms. As has been established, the absorption of carbon dioxide is not the monopoly of green plants and purple sulphur bacteria, but takes place in many heterotrophic microbes. Pathogenic species are not devoid of this capacity too.

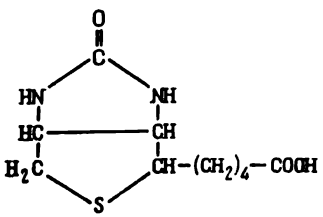
The problem arises, which organisms appeared first, autotrophic

or heterotrophic. S. Vinogradsky, L. Omeliansky, B. Knight, A. Lvov and others consider autotrophic bacteria to be the first organisms. Most scientists adhere to the opinion that the first living organisms were heterotrophs feeding on organic matter. According to this conception, it is considered that anaerobes, microorganisms living in an oxygen-free medium, occurred first, since at that time there was almost no oxygen in the atmosphere of the Earth. The increase in the concentration of the latter was associated with the intensive development of green plants. In the process of evolution autotrophic organisms were produced which began to utilize free oxygen for the purpose of obtaining the necessary energy. Thus, the aerobic type of respiration is inherent in the later period of development of microorganisms.

Vitamins of bacteria. Besides peptones, carbohydrates, fatty acids and inorganic elements bacteria require special substances—vitamins or growth factors which act as catalysts in the biochemical cellular processes, and are structural units for the production of certain enzymes.

In 1901 E. Wildier discovered that yeast contains a substance which he named bios, which has now been shown to consist of three fractions: bios I (meso-inositol), bios II (pantothenic acid and biotin), bios III (thiamine or vitamin B_1).

In 1904 J. Nikitinsky in cultures of mould fungi revealed the presence of organic substances—growth stimulators. For their development most microbes require biotin (vitamin H) which occurs as two isomers: alpha-biotin and beta-biotin.



Biotin

Some microbes do not require a supplement of vitamins to the nutrient medium as they are able to synthesize these compounds themselves. Others grow poorly on vitamin-free media, but their growth is enhanced upon the addition of vitamins. Microbes such as pneumococcus and haemolytic streptococcus cannot be cultivated on completely vitamin-free media.

Influenza bacteria require complex substances found in the blood for their growth: X-factor (haemin) and Y-factor (coenzyme of dehydrogenase).

Vitamins necessary for the growth of bacteria include biotin, vitamins B_1 (thiamine), B_2 (riboflavin), B_3 (pantothenic acid), B_4 (choline), B_5 (nicotinamide), B_6 (pyridoxine), B_7 (haemin), B_8 (inositol), B_9, B_{10}, B_{11} (compounds of folic acid), B_{12} (cyanocobalamine), vitamins K, K_2, etc.

The amount of vitamins in the nutrient medium is expressed in micrograms, and they are required in concentrations varying within the range of 0.001-10μg/l. High concentration of vitamins inhibits the growth of microbes.

Intestinal microflora plays a particular role in supplying animals and man directly with vitamins. Many microorganisms participate in the vitamin metabolism of plants, in enriching food products with vitamins, and in the production of vitamins. At the present time all microbes have been studied comparatively well, and have been classified into certain groups according to their ability to synthesize vitamins.

Importance of inorganic substances in the nutrition of bacteria. Bacteria require inorganic elements. Potassium exerts a catalytic action, and activates enzyme systems. Calcium participates in nitrification, nitrogen fixation by soil microorganisms (*Azotobacter*) and in the production of gelatinase. Phosphorus, sulphur, magnesium, iron are of great importance in the life processes of bacteria. It has been established that iron is found in the respiratory enzymes, and functions as a catalyst in oxidation processes. It is an essential element in the chemical composition of *Mycobacterium tuberculosis*, *Corynebacterium diphtheriae*, colibacillus and other microbes.

Trace elements are incorporated into the structure of the active groups of some enzymes.

THE MECHANISM OF METABOLISM IN MICROORGANISMS

The microbe cell utilizes nutrient substrates for the synthesis of its component parts, for depositing reserve material, for the synthesis of enzymes, pigments, vitamins, toxins and also for obtaining energy needed for its existence.

Already in the first half of the past century L. Pasteur had established that many substances (proteins, hormones, enzymes, and carbohydrates) which play a decisive role in the life processes of animals and plants are optically active and are capable of rotating the plane of polarized light.

He proved that certain species of microorganisms assimilate only one of two optical antipodal cells, the other is not utilized and can be separated in a pure state. Thus, for example, *Penicillium glaucum* uses the dextrorotatory isomer of tartaric acid, *Streptococcus lactis*, the dextrorotatory isomer of lactic acid, etc. The majority of species of colibacillus readily assimilates the levorotatory isomer of leucine.

During metabolism two opposite and at the same time indivisible processes occur: *assimilation* and *dissimilation*, constructive and energy metabolism.

Constructive metabolism (assimilation) proceeds with the absorption of free energy. For this type of metabolism a comparatively small amount of food material used by the cell is expended.

Energy metabolism (dissimilation) serves for the production of energy. For this process a large quantity of nutrients is used up. These two processes cannot be separated, and are in fact interconnected. Thus, for example, products of the incomplete oxidation of a substrate are valuable to the organism not only as energy sources, but as component parts which are used for building up the cell. Metabolism is carried out with the help of enzymes.

ENZYMES AND THEIR ROLE IN METABOLISM

Enzymes, organic catalysts of a highly molecular structure, are produced by the living cell. They are of a protein nature, are strictly specific in action and play an important part in the metabolism of microorganisms.

Enzymes of microbial origin have various effects and are highly active. They have found a wide application in industry and are gradually replacing preparations produced by higher plants and animals.

With the help of amylase produced by mould fungi starch is saccharified and this is employed in beer making, industrial alcohol production and bread making. Proteinases produced by microbes are used for removing the hair from hides, tanning hides, liquefying the gelatinous layer from films during regeneration, and for dry cleaning. Fibrinolysin produced by streptococci dissolves the thrombi in human blood vessels. Enzymes which hydrolyse cellulose aid in an easier assimilation of rough fodder.

Due to the application of microbial enzymes, the medical industry has been able to obtain alkaloids, polysaccharides and steroids (hydrocortisone, prednisone, prednisolone, etc.).

Enzymes permit some species of microorganisms to assimilate methane, butane and other hydrocarbons, and to synthesize complex organic compounds from them. Thus, for example, with the help of the enzymatic ability of yeasts in special-type industrial installations protein-vitamin concentrates (PVC) can be obtained from waste products of petroleum (paraffins), which are employed in animal husbandry as a valuable nutrient substance supplementing rough fodder.

Some enzymes are excreted by the cell into the environment (exoenzymes) for breaking down complex colloid nutrient materials, while other enzymes are contained inside the cell (endoenzymes).

Up to the present time all the 700 known enzymes have been sub-

divided into 6 classes according to the character of catalysis: oxyreductases, transferases, hydrolases, liases, isomerases and ligases.

Depending on the conditions of origin of enzymes there are *constitutive* enzymes which are constantly found in the cell irrespective of the presence of a catalysing substrate. These include the main enzymes of cellular metabolism (lipase, carbohydrase, proteinase, oxydase, etc.). *Adaptive* enzymes occur only in the presence of the corresponding substrate (penicillinase, etc.).

According to chemical properties enzymes can be subdivided into 3 groups:

(1) enzymes composed only of proteins;

(2) enzymes containing in addition to protein metallic ions essential for their activity, and assisting in the combination of the enzyme with the substrate, and taking part in the cyclic enzymatic transformations;

(3) enzymes which contain distinct organic molecules (coenzymes, prosthetic groups) essential for their activity. Some enzymes contain vitamins.

Bacterial enzymes are subdivided into 4 groups:

1. **Hydrolases** which catalyse the breakdown of the link between the carbon and nitrogen atoms, between the oxygen and sulphur atoms, binding one molecule of water (esterases, glucosidases, proteases, amidases, nucleases, etc.).

2. **Transferases** perform catalysis by transferring certain radicals from one molecule to another (transglucosidases, transacylases, transaminases).

3. **Oxidative enzymes** (oxyreductases) which catalyse the oxidation-reduction processes (oxidases, dehydrogenases, peroxidases, catalases).

4. **Isomerases and racemases** play an important part in carbohydrate metabolism. They are found in most species of bacteria. Phosphohexoisomerase, galactovaldenase, phosphoglucomutase, phosphoglyceromutase pertain to the isomerases.

The absorption of food material by the cell is a rather complex process. During diffusion the dissolved substance is transferred from the region of higher concentration outside the cell into the bacterial cell until the concentration becomes the same. The passage of a solvent through the cytoplasmic membrane of bacteria from a region where it is less concentrated to one where it is more concentrated is performed by osmosis. The concentration gradient and osmotic power on both sides of the cytoplasmic membrane are quite different, and depend on the difference in concentration of many substances contained in the cell and nutrient medium. The transfer of dissolved substances from the nutrient medium to the cell can take place by suction together with the solvent if the membrane is sufficiently porous.

It has been established that the cellular membranes are made up

of lipid and protein molecules arranged in a certain sequence. The charged groups of molecules have their ends directed towards the surface of the membrane. On these charged ends the protein layers are adsorbed, composed of protein chains forming a meshwork on the external and internal surfaces of the membrane. The high selectivity which allows the cells to distinguish certain substances from others depends on the presence of enzymatic systems localized on the surface of bacterial cells. Due to the action of these enzymes the insoluble substances in the membrane become soluble.

In the process of bacterial nutrition great importance is attached to exchange adsorption. The active transport of ions takes place due to the difference in charges on the surface of membranes in the cell wall and the surrounding medium of the microorganisms. Besides, the role of transporters, as has been suggested, is performed by liposoluble substances X and Y. Compounds are formed with ions of potassium and sodium (KX and NaY) which are capable of diffusing through the cell wall, while the membrane remains unpenetrable for free transporters.

PROTEIN METABOLISM

Microorganisms for their nutrition, growth and metabolism require various amino acids. Some microbes need one amino acid (for example, enteric fever bacteria require tryptophan) while others require two or more amino acids. There are certain microorganisms (e.g., *Leuconostoc mesenteroides*) which require 17-18 amino acids for their growth.

Many bacteria lack the ability to synthesize amino acids. Usually those species which require vitamins are in need of ready-made amino acids.

Biochemical investigations have established that there are microorganisms which, besides amino acids and vitamins, require substances performing the function of growth stimulators (oleic acid, acetic acid, purine and pyrimidine bases).

Protein metabolism in bacteria proceeds in two phases. The first, the breakdown of protein to the stage of peptones, takes place by means of exoproteinase excreted by the bacterial cell into the external environment. The second breakdown takes place due to the action of endoproteinase which is inherent in all bacteria and is found inside the microbial cell.

The decomposition of protein to the peptone stage takes place in the nutrient medium when the pH is within the range of 7.0-8.0.

Amino acids produced as a result of the action of endoproteinase are subject to deamination with the production of ammonia and X-ketoacid or alcohol, carbon dioxide and ammonia (yeasts), or X-oxyacid and ammonia (lactobacilli), etc.

There is oxidative deamination ($RCHNH_2COOH+{}^1/_2O_2 \rightarrow RCOCOOH+NH_3$), reduced, hydrolytic and intramolecular deamination.

As a result of the breakdown of amino acids the reaction of the medium becomes alkaline due to the production of a weak acid and ammonia.

Besides deamination a widespread phenomenon is *decarboxylation*, especially among the saprophytic bacteria $RCHNH_2—COOH \rightarrow RCH_2NH_2+CO_2$.

Histamine is produced by the decarboxylation of histidine, putrescine—by ornithine, cadaverine—by lysine and tyramine—by tyrosine, respectively.

Some microbes are able to excrete the enzyme tryptophanase by the action of which indole is produced (Fig. 27). Its detection is employed in bacteriological diagnosis. Indole production usually occurs under conditions of starvation of microbes.

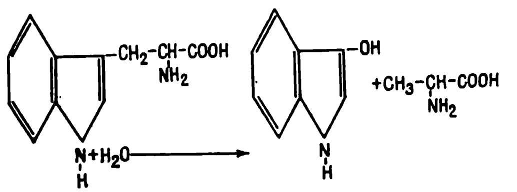


Fig. 27. The cleavage of tryptophan to indole and alanine

Besides the breakdown of proteins, reactions involving protein synthesis also occur. Bacteria require amino acids to build proteins. Bacterial cells satisfy their requirement for amino acids in two ways. Some microorganisms receive them in a ready state, others synthesize amino acids from simple nitrogen compounds. The production of amino acids by microorganisms takes place by binding the future amino acid NH_3 to the carbon framework, while dicarboxylic amino acids, asparaginic and glutamic acids are formed. Other amino acids are produced by transamination $RCHNH_2—COOH+HOOC—CO—COOH \rightarrow RCO—COOH+HOOC—CHNH_2—COOH$. A rather important property of microbes is their ability to synthesize essential amino acids—methionine, tryptophan, lysine.

The synthesis of proteins is performed under the influence of primary information carried by the DNA. An important link in the synthesis of protein is the genetic mediator, the function of which is performed by the template of ribonucleic acid which has been given the name of messenger or information RNA. The synthesis of the chains of RNA according to the DNA template is catalysed by a certain enzyme—RNA-polymerase. The production of protein

takes place in the ribosomes found in the cytoplasm. Ribosomes are made up of equal quantities of protein and RNA (ribosome RNA). On production of RNA on the DNA template, uracil is found in the complementary segment of the RNA chain opposite each adenine in the DNA (Fig. 28). The RNA-mediator thus produced directs

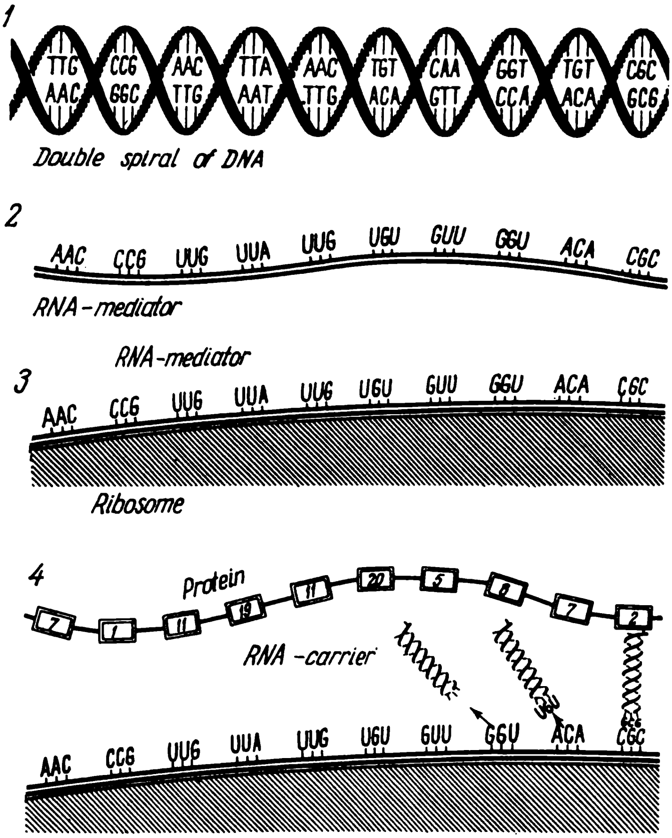


Fig. 28. Protein synthesis on the ribosome surface

protein synthesis. It leaves the cell nucleus and becomes attached to the ribosome. Each amino acid is directed to a specific segment on the RNA-mediator with the aid of an RNA-messenger and an activated enzyme. This was illustrated by M. Nirenberg.

During protein synthesis, the bacterial cell should receive the required amount of amino acids which can be directed by a three-letter code with the help of which all amino acids making up the protein are coded.

It is known that a typical bacterial cell requires not less than 100 enzymes for its normal development and function, although there are some bacteria of the genus *Mycoplasma* (*Mycoplasma gallisepticum*) which require only 40 enzymes for their life activities. Possibly in these simple cells, enzymes are markedly less specific and have a multilateral activity.

Protein metabolism is not autonomic, but is closely connected with carbohydrate metabolism. Pyruvic acid is used for building nitrogen compounds, while dicarbonic acids are active mediators in the biosynthesis of amino acids.

CARBOHYDRATE METABOLISM

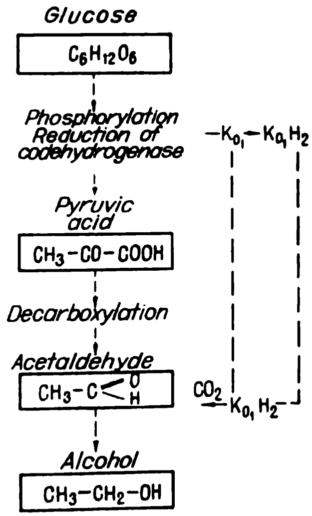
The enzymes (amylase and diastase) which break down carbohydrates hydrolyse starch to glucose and maltose. Amylase is found in many species of microbes, e.g., hay fever, anthrax and diphtheria bacilli, cholera vibrio, and streptococcus. The presence of this enzyme allows the microbe to produce polysaccharides in the cell which serve as reserve food material. Some bacteria possess the enzyme cellulase which breaks down cellulose. A few bacteria are capable of fermenting plant gums—complex polysaccharides, for example agar-agar obtained from sea plants (agar-agar is widely used in the preparation of solid nutrient media). The breakdown of pectin and pectic acid of flax or hemp takes place due to the action of the enzyme pectinase.

Under the influence of maltase, saccharase and lactase entering the bacterial cell, the disaccharides are hydrolysed and break down to monosaccharides which are then fermented. The breakdown of poly- and disaccharides into monosaccharides can take place by phosphorolysis.

During hydrolysis and phosphorolysis of poly- and disaccharides no cleavage of the carbon chain in the carbohydrate molecule takes place and no energy is released, while during fermentation this process occurs and results in the liberation of a large amount of energy. Monosaccharides bound to the phosphoric acid are exposed to fermentative processes. Two molecules of the phosphoric acid combine with the carbohydrate molecule. A series of hexosediphosphoric acids is produced which break down with the cleavage of the six-carbon chain and produce two triose phosphates: phosphoglyceraldehyde and phosphodihydroxyacetone.

As a result of a sequence of reactions the reduction of codehydrogenase takes place, then the produced pyruvic acid through oxidative decarboxylation transforms into acetaldehyde which after receiving hydrogen from the reduced codehydrogenase is converted into alcohol (see diagram on the following page).

Oxidative deamination of amino acids is accompanied by the production of keto acids including pyruvic acid, the oxidation of which



gradually liberates energy for utilization by the organism. The end products of this kind of breakdown are water and carbon dioxide.

Different kinds of microbes cause the breakdown of pyruvic acid by means of different reactions producing unequal amounts of fermentation products. Colibacilli and typhoid bacilli on fermenting one molecule of glucose break it down into two molecules of pyruvic acid from which lactic, acetic and formic acids are then produced. In these cases one may speak of the fermentation of carbohydrates with the production of acids.

$$\underset{\text{pyruvic acid}}{2CH_3-CO-COOH} + H_2O \longrightarrow \underset{\text{lactic acid}}{CH_3-CHOH-COOH} + \underset{\text{acetic acid}}{CH_3-COOH} + CO_2$$

$$\underset{\text{pyruvic acid}}{CH_3-CO-COOH} + H_2O \longrightarrow \underset{\text{acetic acid}}{CH_3-COOH} - \underset{\text{formic acid}}{HCOOH}$$

The breakdown of carbohydrates by microbes is accompanied by an acid reaction, the breakdown of proteins—by an alkaline reaction. Thus in the process of evolution microbes causing fermentation adapted themselves to life in an acid medium, and putrifying microbes—to an alkaline medium. The manifestation of biological antagonism between fermentative and putrifying bacteria plays an important part in nature and in human life. The spoilage of silage, fermented vegetables and milk products can be avoided due to fermentative processes.

Some species of intestinal and typhoid bacteria (coli and paratyphoid) ferment carbohydrates with the production of acid and gas which is a rather important differential character. The production

of gas in this case is due to the breakdown of formic acid to carbon dioxide and hydrogen: $HCOOH \rightarrow H_2 + CO_2$.

Between the energy-yielding and energy-consuming reactions there is an intermediate link of adenosinetriphosphate.

The synthesis of carbohydrates occurs in two ways: by photosynthesis (green and purple bacteria containing pigments of the chlorophyll type in the cytoplasm), and chemosynthesis (most species of bacteria).

The chlorophyll of bacteria is similar to the chlorophyll of green plants (see p. 122). In contrast to plants, bacterial chlorophyll is capable of absorbing a few rays from the infra-red spectrum. It is a complex substance with carotenoid pigments containing 75 per cent of proteins, about 20 per cent of lipids and 5 per cent of chlorophyll. During photosynthesis in purple sulphur bacteria, the reduction of carbon dioxide by the hydrogen of H_2S takes place, while in a number of other purple bacteria reduction is performed by means of the hydrogen of certain organic substances.

Chemosynthesis takes place when carbon is assimilated from carbon dioxide by the oxidation of certain mineral substances. For the assimilation of carbon dioxide hydrogen bacteria use molecular hydrogen, which is oxidized to water.

Heterotrophic microorganisms use carbon from organic compounds which are characterized by their optical activity.

LIPID METABOLISM

Although lipids do not represent an important component in the nutrient substrate they are of certain significance in the life activities of bacteria, and, in particular, they make the microorganisms highly resistant to the harmful factors of the environment.

The majority of bacterial species absorb glycerin quite well, which serves as an energy source and as plastic material for the synthesis of the component structures of the microbial cell. Tubercle bacilli and other acid-fast microbes use glycerin for the synthesis of lipids. As is known, lipid inclusions in bacterial cells function as reserve food material.

Metabolic processes are carried out with the help of lipase and other lipolytic enzymes bound firmly to the cell cytoplasm. An important role is played in the breakdown of lipids by coenzyme A, the thiol group of beta-mercaptoethylamine.

Numerous bacteria use methionine as a carbon source for the synthesis of lipids. Mykolic acid of the lipids of corynebacteria is synthesized in the bacterial cell by condensation of two molecules of palmitic acid and reduction of the intermediate beta-ester. The synthesis of mykolic acid in mycobacteria takes place in a similar way.

Besides nitrogen and carbon, microorganisms require ash elements (sulphur, phosphorus, potassium, calcium) and trace elements (boron, molybdenum, zinc, manganese, cobalt, nickel, copper, iodine, bromine, etc.) for the synthesis of the bacterial cell.

One of the most important elements in the bacterial cytoplasm is sulphur. It takes part in synthetic reactions as the compound R-SH. This reduced form of sulphur has a high reactivity and is easily dehydrated with the subsequent transformations into the R-S-S-R group, and then into more complex compounds which are reduced upon hydration. Due to this process the oxidation-reduction potential in the cytoplasm of microorganisms can be controlled. The reduction of a sulphate takes place in the following way: $-SO_4 \rightarrow -SO_3 \rightarrow -SO_2 \rightarrow -SO \rightarrow H_2S$.

Some species (sulphur bacteria, thiobacteria) utilize reduced sulphur compounds (hydrogen sulphide and even sulphur). Pathogenic bacteria use sulphur as a sulphohydryl group (R-SH).

Phosphorus is contained in nucleic acids, in many enzymes, in different phospholipids and other organic compounds in the form of P_2O_5. It does not combine directly with carbon but forms a bond through the oxygen atoms. In the process of oxidation the energy accumulated in the cytoplasm of microbial cells is liberated. Adenosinetriphosphoric acid and adenosinediphosphoric acid play an important part in the energetic metabolism of the microbial cell. The former is rich in energy (charged), whereas the latter is a poor source of energy (discharged). Phosphorus is contained in the most important compounds of the cell cytoplasm (nucleoproteins, phospholipids, prosthetic groups of most of the two-component enzymes). Five per cent of the dry matter of bacterial cells consists of phosphorus in the form of P_2O_5.

Cations and anions of many metals (magnesium, calcium, potassium, iron, etc.) taking part in the synthesis of cellular substance are necessary for the normal development of microorganisms. Thus, for example, iron is found in haemins which are prosthetic groups for a number of enzymes (cytochromes). A deficiency or excess of iron in the nutrient medium causes a different production of the toxin by the diphtheria bacilli. Trace elements take part in the synthesis and activation of enzymes. Molybdenum and boron are necessary for nitrogen-fixing bacteria.

PRACTICAL USE OF THE FERMENTATIVE PROPERTIES OF MICROBES

The widespread and theoretically founded application of microbiological processes in the technology of industries involving fermentation, treatment of flax, hides, farming and canning of many

food products became possible only in the second half of the 19th century. From the vital requirements of a vigorously developing industry, especially of the agricultural produce processing industry, there arose a need for a profound study of biochemical processes. The investigations by L. Pasteur in this field were to a great extent prepared by the development of industry, organic chemistry and other sciences.

Of great importance in medical microbiology is the utilization of the specific fermentative capacity of pathogenic bacteria for the determination of their specific properties. Many bacteria ferment carbohydrates producing acid or acid and gas, while proteins are fermented with the production of indole, ammonia, hydrogen sulphide, etc.

Fermentative properties of microbes are used in the laboratory diagnosis of infectious diseases, and in studying microbes of the soil, water and air.

It has been established that there is a certain interrelationship between the degree of parasitism and fermentative activity of pathogenic microbes. The more distinct the parasitism in the microbe, the lower its fermentative activity. However, it should be noted that such an interrelationship between fermentative activity and parasitism is not a general rule. Some bacteria (cholera vibrio, causative agent of plague) have rather distinct biochemical properties, and at the same time belong to the most pathogenic species.

RESPIRATION IN MICROORGANISMS

Respiration in bacteria is a complex process which is accompanied by the liberation of energy required by the microorganism for the synthesis of different organic compounds. Many microbes similar to vertebrates and plants utilize the molecular oxygen in the air for respiration.

The concept of respiration as a process of oxidation of organic substances with the production of energy has undergone considerable changes due to the discovery of anaerobic microbes unable to exist in the presence of oxygen. L. Pasteur established that the energy necessary for the life activity of some species of microbes is obtained in the process of fermentation (liberation of energy without the participation of oxygen).

All microbes according to type of respiration can be subdivided into aerobic and anaerobic. Between them there are intermediate forms:

1. Obligate aerobes which develop well in an atmosphere containing 21 per cent of oxygen. They grow on the surfaces of liquid and solid nutrient media (cholera vibrio, sarcinae, tubercle bacilli, etc.).

2. Microaerophilic microbes which require a small amount of oxygen (up to 1 per cent)—lactic-acid bacteria, etc.

3. Facultative aerobes which can reproduce even in the absence of molecular oxygen (the majority of pathogenic and saprophytic microbes).

4. Capnophilic bacteria requiring a reduced concentration of oxygen and an increased amount of carbon dioxide (brucellae of the bovine type, etc.).

5. Obligate anaerobes for which the presence of molecular oxygen is a harmful growth-inhibiting factor (causative agents of tetanus, botulism, gas gangrene, etc.).

Aerobic bacteria in the process of respiration oxidize different organic substances (carbohydrates, proteins, lipids, alcohols, organic acids and other compounds). During complete oxidation of one gram-molecule of glucose, 688.5 Cal are liberated which corresponds to the potential energy store accumulated in the carbohydrate molecule during its photosynthesis in green plants from carbon dioxide and water

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + 688.5 \text{ Cal}$$

During incomplete (partial) aerobic oxidation, less energy is released corresponding to the degree of oxidation

$$2C_6H_{12}O_6 + 9O_2 \longrightarrow 6C_2H_2O_4 + 6H_2O + 493 \text{ Cal}$$

A typical representative of the facultative aerobes is the colibacillus which in a carbohydrate medium begins to develop first as an anaerobe breaking down the carbohydrates by fermentation. Then it begins to utilize oxygen and grows like an aerobe, oxidizing the products of fermentation (lactic acid) farther to carbon dioxide and water. Facultative aerobes have a considerable advantage, as they can live in aerobic and anaerobic conditions.

Respiration in anaerobes takes place by fermentation of the substrate with the production of a small amount of energy. In the fermentation of one gram-molecule of glucose considerably less energy is produced than during aerobic respiration

$$C_6H_{12}O_6 \longrightarrow C_3H_6O_3 + 225 \text{ Cal}$$
$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2 + 27 \text{ Cal}$$
$$C_6H_{12}O_6 \longrightarrow CH_3COOH + 15 \text{ Cal}$$

According to Dudorov's hypothesis the limiting factor of anaerobic growth is energy and of aerobic growth—carbon. Thus, for example, during glycolysis a low energy yield is determined by an incomplete breakdown of the substrate because of the deficiency of electron acceptors. During respiration an excess of oxygen, playing the part of an electron acceptor, and competition with numerous other microorganisms cause the carbon to transform into carbon dioxide not only by way of "useful" oxidation, i.e., liberation of biologically absorbable energy, but also as a result of uncontrolled

oxidation. As has been established, the assimilation of carbon takes place slowly in anaerobic cells, and relatively rapidly in aerobic cells.

The mechanism of anaerobic respiration takes place in the following way. If carbohydrates make up the oxidizing substrate, then preliminarily they are broken down with the help of auxiliary enzymes. Thus, for example, glucose is phosphorylated employing ATP and ADP. As a result, hexosediphosphate is produced which under the influence of the enzyme aldolase breaks down into two components: phosphoglyceraldehyde and dioxyacetone phosphate. The latter under the influence of oxyisomerase is transformed into phosphoglyceraldehyde and later on after a sequence of reactions produces pyruvic acid. This stage is the last in the anaerobic phase of transformation of carbon. The later stages are specific and are completed with the production of end products.

Anaerobic processes include alcohol fermentation by yeasts, lactic acid fermentation by lactobacilli, and butyric acid fermentation by butyric acid clostridia.

Anaerobes ferment mostly nitrogen-free compounds causing fermentation. However, there is no sharp boundary between the aerobic and anaerobic types of respiration. Thus, for example, yeasts can change the anaerobic type of respiration to aerobic respiration. First of all, they break down sugar into alcohol and carbon dioxide, and during increased aeration glucose is broken down into water and carbon dioxide.

The presence of obligate anaerobes explains the rather great adaptability of living things and the completeness of the cycle of substances in nature.

It has been established by investigations that the respiration in bacteria takes place under the influence of enzymes of the oxidase and dehydrogenase types, which have a marked specificity and a multilateral activity. The oxidase and dehydrogenase processes of respiration are closely interconnected, supplementing each other, but at the same time differing in biological role and in enzymes.

V. Palladin was the first to demonstrate that there is no fundamental difference between aerobic and anaerobic oxidation. Aerobic respiration is the process of oxidation when oxygen serves as the hydrogen acceptor. Anaerobic respiration is that process of oxidation when any substance except for oxygen may be the hydrogen acceptor. During aerobic respiration the oxidase system carries out the oxidizing process by the reaction of oxygen with the hydrogen of the organic substance.

According to Bach's theory, cells contain an oxidizing system made up of oxygenase which with molecular oxygen produces peroxides and peroxidases transporting the activated oxygen to the oxidized substrate.

According to A. Bach, the activation of oxygen is related to the

production of peroxides with unsaturated organic substances. By means of energy obtained from the unsaturated substance, molecules of oxygen leave their inert condition and become active.

This reaction takes place in two phases. In the first phase, molecular oxygen is activated and combines with the organic substance (A), producing peroxides. This can be represented schematically

$$A + O_2 \longrightarrow AO_2 \text{ or } A\begin{matrix} \diagup \\ \diagdown \end{matrix}\begin{matrix} O \\ \| \\ O \end{matrix}$$

In the second phase, the peroxide compound AO_2 releases the excess of oxygen to the oxidized substrate B, which could otherwise not be oxidized by oxygen

$$AO_2 + B \longrightarrow AO + BO$$

Later, the intermediate oxidized compound AO produced during the second phase receives oxygen and transforms into the peroxide form AO_2, and is reduced again releasing its excess of oxygen to the substrate B. This process continues until all of the substrate is oxidized.

Aerobic bacteria are relatively stable to peroxides as they are capable of producing peroxidase and catalase enzymes which decompose peroxides. Peroxidase upon decomposing hydrogen peroxide oxidizes various compounds. Catalase breaks down hydrogen peroxide into oxygen and water

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

According to O. Warburg the activator of oxygen is the respiratory enzyme similar in chemical composition to haemin. It is cytochromoxidase—a protein complex with ferroporphyrin which is found together with cytochromes in animal and plant cells and in aerobic microbes. The presence of three types of cytochrome (a,b,c) with characteristic absorption bands has been established. Cytochrome contains iron, and is composed of histidine, lysine, and many other amino acids.

Upon oxidation and reduction of cytochrome the valence of iron in the ferroporphyrin group changes.

The ferroporphyrin group of catalase upon decomposition of hydrogen peroxide changes the valence of iron

$$4Fe^{+++} - \text{catalase} + 2H_2O_2 \longrightarrow 4Fe^{++} - \text{catalase} +$$
$$+ 4H + 2O_2 \longrightarrow 4Fe^{+++} - \text{catalase} + 2H_2O + O_2$$

Aerobes (blue pus, hay fever, anthrax, tubercle bacilli, cholera vibrio, etc.) contain all three cytochromes: a, b, and c. Facultative aerobes (colibacillus, enteric fever, paratyphoid and dysentery bacteria, streptococcus, etc.) contain only one or two cytochromes. Anaerobes do not contain cytochrome.

The intensity of the processes of aerobic respiration depends on the age of the culture, temperature and nutrient substrates. Actively growing cultures use 2,500-5,000 cu mm of oxygen per 1 mg of dry matter of bacteria per hour, while starved cultures or cultures completely deprived of nitrogen nutrients require only 10-150 cu mm of oxygen. A young culture produces considerably more heat energy than it uses for its synthetic and other life processes. A certain part of this energy is released into the environment. For instance, the colibacillus in the process of assimilation uses 31 per cent of the energy released, blue pus bacteria—28 per cent, *Proteus vulgaris*—20 per cent and salmonellae of enteric fever—12 per cent. The production by some microbes of an excess of heat energy in manure, turf and garbage can cause spontaneous heating and spontaneous combustion.

In manure and garbage dumps due to the effect of the high temperature produced by thermophilic microbes, the eggs laid by flies and also the eggs of worms are unable to develop.

Increased respiration and an increased metabolism depend on the rate of cell reproduction, on the increase of the protein synthesis in the cell, which causes an increase in the reduction properties of the medium in which the microbes develop.

According to G. Wieland oxidation is the removal of hydrogen, reduction is the addition of hydrogen.

According to the theory of Palladin-Wieland, respiration in bacteria takes place under the influence of dehydrogenase, which causes the activated hydrogen to be transported from the oxidizable substance (hydrogen donor) to the hydrogen acceptor. The reaction can be illustrated thus: $AH_2 + B \xrightarrow[\text{dehydrogenase}]{\text{enzyme}} A + BH_2$

As can be seen the hydrogen donor AH_2 is oxidized, while the hydrogen acceptor B is reduced to BH_2.

The function of the hydrogen acceptor can be performed by the oxygen of the air, and by organic substances capable of oxidation and reduction (codehydrogenase, yellow pigments and possibly substances found in bacterial pigments).

The transformation of cuprous chloride into cupric chloride ($CuCl \rightarrow CuCl_2$) is also an oxidation reaction. As a result the copper atom loses its negative electrical charge, and univalent copper becomes bivalent. Thus all the mechanisms by means of which the process of oxidation takes place include the loss of one or more electrons, while reduction—the addition of one or more negative electrons. The reaction is shown in Fig. 29.

Thus the processes of respiration in bacteria are very complex and represent a long chain of a sequence of oxidation-reduction reactions with the participation of many enzyme systems transporting the electrons from the system of the most negative potential to the system of the most positive potential. During gradual and

fractional liberation of energy in respiration and during intermediate transport of hydrogen, the activity of cellular reactions increases (Fig. 29). The biochemical mechanisms of respiration are described in detail in biochemistry textbooks.

The habitat of microorganisms greatly influences the character of respiration. Thus, for example, upon cultivating the cholera-like vibrio in a medium containing glucose, its aerobic respiration can be decreased as a result of which it acquires the properties of a facultative anaerobe. Yeasts are also capable of changing their type of respiration depending on the presence or absence of oxygen.

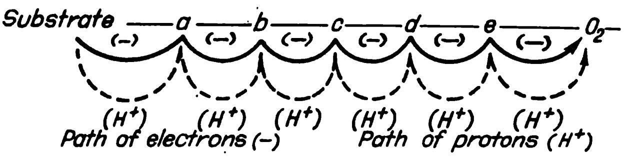


Fig. 29. Diagram of the oxidation of the substrate by transporting electrons

G. McLeod explained that the toxic effect of oxygen on anaerobes is due to the production of hydrogen peroxide in the presence of oxygen. Anaerobes are unable to produce catalase. Only H_2O_2 but not oxygen itself is toxic. However, this cannot be a complete explanation. Anaerobes can grow if there is oxygen in the medium, which does not kill microbes, but only inhibits their life activities. Upon the addition of reducing agents to the medium, the microbes begin to grow, as reducing agents lower the oxidation-reduction potential. Glucose and other reducing substances act in the same way.

V. Engelhardt considers that in the presence of a high oxidation-reduction potential, the inactivation of vitally important enzymes takes place. Anaerobes then lose their ability to feed normally, to carry out constructive processes and perish from starvation, and not from intoxication by oxygen or H_2O_2.

The oxidation-reduction potential (rH_2) is one of the factors on which the oxidation-reduction reactions in the nutrient medium depend. The oxidation-reduction potential expresses the quantitative character of the degree of aerobiosis. It becomes minimal upon saturating the medium with hydrogen, and maximal upon saturating the medium with oxygen. M. Clark proposed to designate the unit of the oxidation-reduction potential as rH_2—the negative logarithm of the partial pressure of gaseous hydrogen. The range of rH_2 from 0 to 42.6 characterizes all degrees of saturation of an aqueous solution with hydrogen and oxygen. Aerobes exist within the limits of rH_2 from 14 to 20 and more, facultative aerobes from 0 to 20 and more, and anaerobes from 0 to 12.

Aerobes are adapted to existence at a higher oxidation-reduction potential, anaerobes—at a lower rH_2. Anaerobes are not passive microorganisms, and they themselves cause the low rH_2 in the medium. Seeded cultures of anaerobes prior to reproduction lower the rH_2 from 20-22 to 1-5. Thus anaerobes are characterized by a rather marked capability to adapt the medium to their requirements. Aerobes have these properties too, and guard themselves from an excess of oxygen by a reduction barrier.

In nature, frequently marked changes from aerobic conditions to anaerobic take place. Thus, for example, after a rainfall the anaerobic conditions take the place of the previously aerobic conditions in the soil. When organic substances (plant and animal cells) enter the soil, reduction conditions occur which are produced due to the active absorption of oxygen by aerobes and also due to their excretion of reducible substances. Microorganisms in contrast to higher organisms rather quickly adapt themselves to sharp changes in oxidation-reduction conditions.

Upon controlling the oxidation-reduction potential of the nutrient medium, conditions can be obtained for the growth of anaerobes in the presence of oxygen by lowering the rH_2, and also by cultivating the aerobes in anaerobic conditions by increasing the rH_2 of the medium.

The oxidation-reduction potential drops sharply when the bacterial culture dies, when it is lysed by a phage and when it is affected by lysozyme.

When preparing nutrient media the composition of the nutrient energy-yielding material, the reaction of the medium (pH), and its oxidation-reduction potential (rH_2) are all taken into consideration.

In contrast to bacteria whose respiration represents a sequence of oxidation-reduction processes, respiration in viruses takes place as a result of the accumulation and expenditure of energy. Nucleic acids make up the energy acceptor in viruses. This was proved by introducing ATP into the nutrient medium which sharply increased the intensity of viral multiplication. Upon infecting white mice with the influenza virus, the amount of ATP in the lungs decreased, as part of this compound was absorbed by the influenza virus.

THE PRODUCTION OF PIGMENTS BY MICROORGANISMS

Some species of bacteria and fungi living in the soil, water and air are capable of producing pigments (see Fig. 117, *8*). Colonies (aggregations of microbes, see p. 99) of pigment-producing microbes on solid media are coloured red (*Bacterium prodigiosum*, actinomycetes, yeasts), pink (*Micrococcus*), gold (*Staphylococcus aureus*),

white (*Staphylococcus albus*), blue (blue pus bacterium), violet (*Chromobacterium violaceum*), black and brown (yeasts and fungi). Sarcina colonies are yellow, lemon and gold-coloured. There are microorganisms which produce two and more pigments.

The production of pigments takes place in the presence of an adequate access to oxygen, at a temperature of 20-25°C and in subdued sunlight.

Pigments can be subdivided into those which are soluble in water (blue pus bacterium, bacterium of blue milk), soluble in alcohol and insoluble in water (*Bacterium prodigiosum*, *Staphylococcus aureus*), insoluble in water and alcohol (azotobacter, black and brown pigments of yeasts and moulds), and also those which are chromopaired (entering the environment) and chromophoric (found in the cytoplasm, vacuoles and cell wall).

Pigment production in microbes has a certain physiological importance. Possibly in the process of respiration pigments function as hydrogen acceptors, give protection from natural ultraviolet radiation and take part in reactions of synthesis, and also have an antibiotic action.

LUMINESCENCE IN MICROORGANISMS

Luminescence constitutes a certain form of liberation of energy in oxidation processes. Luminescence is more intensive, the greater the supply of oxygen to the bacteria.

The luminescence of meat, fish scales and other substances was noticed by Aristotle (384-322 B.C.). Luminescent microorganisms sometimes invade the bodies and muscles of small crustaceans and cause a bright luminescence of these animals in the dark on the sea shores. Luminescent termites, ants and spiders have been discovered, and it has been suggested that the source of light in these insects comes from luminescent bacteria. Some fish have developed special organs for maintaining luminescent bacteria, as symbionts which serve as a light source.

In the beginning of the 20th century H. Molisch suggested the use of luminescent bacteria in safety lamps for powder cellars. Luminescent bacteria are quite sensitive indicators of the presence of molecular oxygen. M. Beijerinck employed luminescent bacteria as an indicator when studying the giving off of oxygen in the process of photosynthesis.

Luminescent bacteria were named photobacteria (Gr. *photos*—light). These include a large group of physiologically similar though morphologically different bacteria (cocci, rods, vibrions). They are either Gram-negative or Gram-positive, nonsporulating aerobes.

The majority of luminescent bacteria are obtained from sea water. They do not cause putrefication, although they grow well on fish and meat substrates, and are cultivated on ordinary media.

The optimal temperature for the growth and luminescence of most species is between 15 and 18°C. Some species develop well at 30-37°C and in a 3 per cent salt concentration. However, there are species which are luminescent in a medium containing 0.5-0.7 per cent NaCl.

The luminescent system of bacteria is associated with unimpaired live cells. The decrease of salts in the medium, the addition of sulphonamides, osmotic cytolysis, mechanical grinding, supersonic vibrations, slow autolysis, extraction by various solvents—all of these factors disturb not only the ability to produce luminescence, but the life activity of the cell. However, extracts emitting light in the dark have been obtained from some organisms.

Luciferin and the enzyme *luciferase* have been isolated from separate extracts.

A typical representative of the photogenic microbes is *Photobacterium phosphoreum* (see Fig. 117, *6*), a nonmotile coccus-like bacterium which does not liquefy gelatin. It develops at 28°C, and at temperatures higher than 30°C growth ceases. Within the photogenic group of bacteria no pathogenic species for man have been established.

ODOROPHORIC BACTERIA

Microorganisms have been revealed which are capable of excreting volatile substances during their life activities. They produce ethyl acetate and amyl acetate ethers.

The aromatic properties of wine, milk products, soil, hay and other substances depend on the activity of certain species of microbes. The odorophoric bacteria include *Leuconostoc citrovorum* employed in the milk industry. It gives an aroma to milk products, especially butter.

* * *

Microbes are capable of producing electric energy as they are electronegative colloids. Vitamins and enzymes may be produced with the help of certain species of microorganisms. Some pathogenic representatives excrete toxic substances (toxins) for man and animals (see. p. 175).

REPRODUCTION AND GROWTH OF MICROORGANISMS

Reproduction in microbes constitutes the ability of self-multiplication, the increase in the number of individuals per unit volume. The growth of microorganisms represents the increase of the mass of bacterial cytoplasm as a result of the synthesis of cellular material.

Bacteria reproduce by simple transverse division, vegetative reproduction (Fig. 30), which occurs in different planes and produces many kinds of cells (clusters, chains, pairs, packets, etc.). They also reproduce by budding, by means of the cleavage of segmented filaments, by reproducing cells similar to spores, by producing minute motile conidia, by conjugation (Fig. 31), which brings us closely

Fig. 30. Ultrathin section of a dividing *E. coli* cell

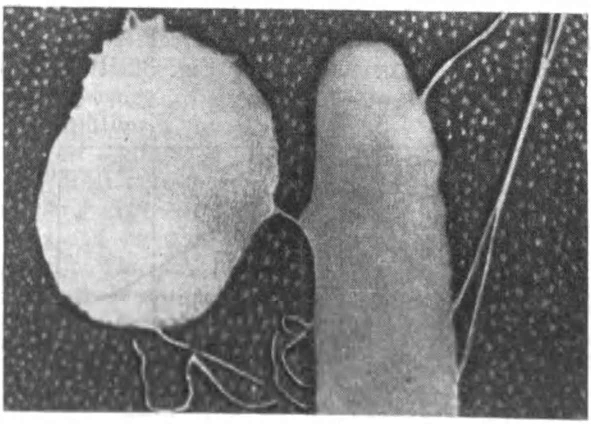


Fig. 31. Conjugation in *E. coli*

to the concept of sexual reproduction in bacteria (see p. 164). Actinomycetes and many fungi (phycomycetes, ascomycetes, etc.) reproduce predominantly by sporulation.

The transverse division of bacteria is not only a process of cell division of one mother cell into two equal daughter cells, but represents a constant separation of daughter cells from the mother cell, the former in their turn become mother cells. After a certain number of generations, the mother cells age and perish. This explanation has annulled the metaphysical concept of "bacterial immortality".

The rate of cell division differs among bacteria. It depends on the species of microbe, the age of the culture, on the nutrient medium, temperature, concentration of carbon dioxide and on many other factors.

The length of the generation of *E. coli*, *Clostridium perfringens*, *Streptococcus fuecolis* is 15 minutes, while for the cells of a mammalian tissue culture it is 24 hours. Thus, bacteria reproduce almost 100 times faster than a tissue culture. The increase in the number of cells can be expressed in the following way:

1—2—4—8—16—32—N number of cells

0—1—2—3—4—5—n number of generations.

The total amount of bacteria (N) after n generations will be equal to 2^n per cell of seeded material. If we take the original amount of bacteria inoculated into the nutrient medium as a single individual, and the time for one division as 30 minutes, then theoretically the total amount of bacteria produced per 24 hours would equal $N=2^{48}$. Upon division every 20 minutes, in 36 hours the microbial mass will be equal to 400 tons. Thermophilic microbes divide even more rapidly.

However, in natural as well as in artificial conditions, the reproduction of bacteria is of a considerably smaller scale. It is limited by the effect of a number of environmental factors. Reproduction in bacteria conforms to certain laws. Fig. 32 illustrates schemati-

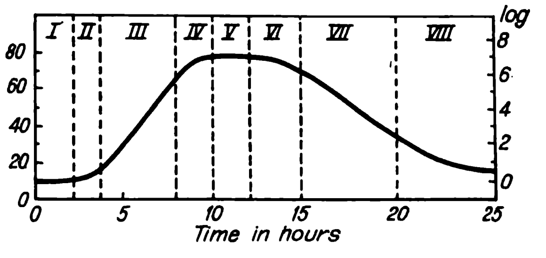


Fig. 32. Graph of the reproduction of bacteria

cally the rate of reproduction in arbitrary units, and the size of the bacterial population expressed as the logarithm of the numbers of live cells per millimetre of the medium.

There are eight principal phases of reproduction which are designated on the diagram by Roman numerals.

1. *An initial stationary phase* represents the time from the moment of seeding the bacteria on the nutrient medium. Reproduction does not occur in this phase. The length of the initial stationary phase after seeding is 1-2 hours.

2. *The lag phase of reproduction* during which bacterial reproduction is not intensive, while the growth rate is accelerated. The second phase may last almost two hours.

3. *Phase of logarithmic growth* which is characterized by a maximal division rate and decrease in cell size. The length of this period ranges from 5 to 6 hours.

4. *Phase of negative growth acceleration* during which the rate of bacterial reproduction ceases to be maximal, and the number of dividing cells diminishes. This phase lasts almost two hours.

5. *A maximal stationary phase* when the number of newly produced bacteria is almost equal to the number of dead organisms. This phase continues for two hours.

6. *Accelerated death phase* during which the equilibrium between the stationary phase and the bacterial death rate is interrupted. This continues for 3 hours.

7. *Logarithmic death phase* when the cells die at a constant rate. This continues almost 5 hours.

8. *Decelerated death-rate phase* in which those cells which remain alive enter a dormant state.

The length of these phases is arbitrary, as it can vary depending on the species of bacteria and the conditions of cultivation. Thus, for example, the colibacilli divide every 15-17 minutes, salmonellae of enteric fever—every 23 minutes, pathogenic streptococci—every 30 minutes, diphtheria bacilli—every 34 minutes and tubercle bacilli—every 18 hours.

Besides the ability to reproduce and to grow, the bacteria vary in *changes during different stages of growth*, maturation and aging. These changes can be seen during *the normal cycle* of individual bacterial growth. The development cycle depends on the nature of the organisms, their complexity and sequence of growth.

The most simple development cycle is found in the coccal-shaped bacteria which is characterized by the growth of the cell and then its subsequent division.

Rod-shaped nonsporulating bacteria have a development cycle similar to that of the cocci. The young cells increase in size, reach a maximum and then divide transversely into two daughter cells which go through the same cycle. Spore formation is included in the development cycle in bacilli under certain conditions.

Chlamydobacteria have a more complex development cycle. Their cells transform into long filaments, some of which produce special reproductive organs, gonidia, which germinate and give rise to new cells and filaments.

Actinomycetes have two different development stages: (1) a vegetative growth stage which is characterized by the production of mycelia; (2) a fruiting stage with the production of spores on spiral or straight branches—sporophores.

Mycobacteria are characterized by a relatively complex development cycle. The vegetative rod-shaped cells are replaced by oval or round-shaped microcysts. The cells form fruiting bodies with specially shaped fruiting stems.

MAIN PRINCIPLES OF THE CULTIVATION OF MICROORGANISMS

Bacterial cultivation. In laboratory conditions microorganisms can be grown in nutrient media in incubation chambers maintained at a constant temperature. According to the type of heating, incubation chambers can be subdivided into electric, gas and kerosene. Each incubation chamber has a thermoregulator which maintains a constant temperature. Temperature conditions are of great importance for the growth and reproduction of bacteria. In relation to conditions of temperature all microorganisms can be subdivided into three groups: psychrophilic (Gr. *psychros*—cold, *philein*—love), mesophilic (Gr. *mesos*—intermediate), thermophilic (Gr. *thermos*—warm), see Table 4.

Table 4

Differentiation of Microbes in Relation to Temperature Conditions

Group of microbes	Temperature limits for reproduction of microbes			Habitat
	optimum	minimum	maximum	
Psychrophilic	+10-20°C	—10-+10°C	25-30°C	Cold seas and oceans, polar soils, permanently frozen zones
Mesophilic	20-40°C	10-25°C	40-45°C	Bodies of animals and man
Thermophilic	50-60°C	25-45°C	70-80°C	Top layers of soil, hot springs, manure, turf, cotton-wool wastes

Of great importance in the life activities of bacteria is the concentration of hydrogen ions in the nutrient medium, i.e., pH, which is expressed by the negative logarithm of the concentration of hydrogen ions. The pH characterizes the degree of acidity or alkalinity, from extremely acid (pH 0) to extermely alkaline (pH 14) conditions.

During evolution each microbial species adapted itself to existence within certain limits of hydrogen ion concentration beyond the range of which its life processes are unable to take place. It has been suggested that pH influences the activity of enzymes. Depending on the pH, weak acids in an acid medium occur as molecules, in an alkaline medium as ions. Saprophytes can live in conditions

within a wide range of a pH from 0.6 to 11.0, while pathogenic species of microbes grow within certain limits of hydrogen ion concentration cited in Table 5.

Nutrient media should be easily assimilable, should contain a known amount of nitrogen and carbohydrate substances, vitamins, a required salt concentration, they should be isotonic, sterile and should have buffer properties, an optimal viscosity and a certain oxidation-reduction potential.

During the whole history of microbiology nutrient media have gradually been perfected. Before L. Pasteur only infusions and decoctions were used as media for growing microbes. L. Pasteur and C. Nägeli introduced nonprotein media for the cultivation of microbes. R. Koch and F. Loeffler employed meat broth, peptone and sodium chloride for growing microbes. This medium is a meat-peptone broth from which meat-peptone agar is prepared by adding 1-2 per cent industrial agar.

Agar-agar (in Malayan—jelly) is a complex organic substance received from marine algae. It is composed of gelose (70-75 per cent), water (11-22 per cent), ash (2-4 per cent), total nitrogen (0.4-0.9 per cent), amino nitrogen (0.03-0.09 per cent). The main or gelling substance of agar is composed of a calcium salt, acid ether, sulphuric acid and a carbohydrate-polysaccharide complex which is composed of different proportions of arabinose, glucose, galactose, etc. Agar-agar melts in water at 80-86°C and solidifies at 36-40°C.

Because of the ability of agar-agar upon cooling to give the nutrient medium a solid gel consistency, and due to its high resistance towards the microbial enzymes it has received wide application in bacteriological techniques for preparing semisolid, solid and dry nutrient media.

For the preparation of nutrient media M. Hottinger suggested the use of products of the tryptic breakdown of proteins which do not contain peptones, but contain the low polypeptides and free amino acids. L. Martin employed papain as an enzyme for the breakdown of proteins. In recent years all the essential amino acids and vitamins used for the cultivation of bacteria have been obtained in a pure state.

Nutrient media can be subdivided into three main groups:

I. *Ordinary* (*simple*) *media* which include meat-peptone broth, meat-peptone agar, etc.

II. *Special media* (serum agar, serum broth, coagulated serum, potatoes, blood agar, blood broth, ascitic broth, ascitic agar, etc.).

Quite often *elective media* are employed in laboratory practice in which only certain species of bacteria grow well, and other species either grow poorly or do not grow at all. *Enriched media* are also employed in which the species of interest to the scientist grows more intensively and more rapidly than the accompanying bacteria Thus, for example, on Endo's medium (elective) the growth of

Table 5

Optimal pH Values at Which Certain Pathogenic Microbes are Cultivated

Name of microbe	pH of medium
Staphylococcus	7.2-7.4
Streptococcus	7.2-7.6
Pneumococcus	7.2-7.6
Meningococcus	7.2-7.5
Gonococcus	7.2-7.6
Pasteurella pestis	6.9-7.0
Causative agent of tularaemia	6.7-7.4
Brucella	6.8-7.2
Actinobacillus mallei	6.4-6.8
Pseudomonas pseudomallei	6.8-7.2
Salmonella typhosa	6.8-7.2
Vibrio cholerae	7.6-8.0
Influenza bacterium	7.3-7.5
Bacilli of anthrax	7.2-7.6
Clostridium tetani	7.0-7.5
Clostridia of gas gangrene	6.0-8.0
Clostridium botulinum	7.4-7.6
Corynebacterium diphtheriae	7.2-7.6
Listeria	7.0-7.2
Mycobacterium tuberculosis	5.8-8.0
Borrelia recurrentis	7.2-7.4
Leptospira	7.2-7.4
Rickettsia	7.4-7.8

the Gram-positive microbes is inhibited, while alkaline peptone water and alkaline meat-peptone agar serve as enriched media for the cholera vibrio. Nutrient media containing certain concentrations of penicillin are elective for penicillin-resistant strains of bacteria, but unfavourable for penicillin-sensitive strains.

III. *Differential diagnostic media*: (1) media for the determination of the proteolytic action of microbes (meat-peptone gelatine); (2) media for the determination of the fermentation of carbohydrates (Hiss media, etc.); media for the differentiation of bacteria which do and do not ferment lactose (Ploskirev, Levine, Drigalsky, Endo, etc.); (3) media for the determination of haemolytic activity (blood agar); (4) media for the determination of the reductive activity of microorganisms; (5) media containing substances assimilated only by certain microbes.

Besides, in laboratory practice *conservation media* are used. They are used for primary seeding and transportation of the material under test. They prevent the death of pathogenic microbes and enhance the inhibition of saprophytes. This group of media includes a glycerin mixture composed of two parts 0.85 per cent

salt solution, 1 part glycerin and 1 part 15-20 per cent acid sodium phosphate, and also a glycerin preservative with lithium salts, a hypertonic salt solution, etc.

At present many nutrient media are prepared commercially as dry powders. They are convenient to work with, are stable, and quite effective.

Nonprotein media are widely used for the cultivation of bacteria, on which many heterotrophic microbes including pathogenic species grow well. The composition of these media is complex and includes a large number of components.

When cultivating in synthetic media, the use of the method of radioactive tracers has permitted a more detailed differentiation of microbes according to the character of their biosynthesis.

In consistency nutrient media may be solid (meat-peptone agar, meat-peptone gelatine, coagulated serum, potato, coagulated white of the egg), semisolid (0.5 per cent meat-peptone agar) and liquid (peptone water, meat-peptone broth, sugar broth, etc.)

On solid nutrient media microbes form *colonies* of different shapes and sizes which are aggregations of individuals connected by bands of cytoplasm providing for a certain structure of bacterial groupings. The colonies may be flat, convex, dome-shaped, or pitted; their surface, smooth (S-forms), rough (R-forms), ridged, or bumpy; their edges may be straight, serrated, fibrous or tasseled. The shape of the colonies also differs: e.g., round, rosette-shaped, star-shaped, tree-like (Figs. 33-34). According to their size the colonies may be

Fig. 33. Colonies of a different structure (top view)

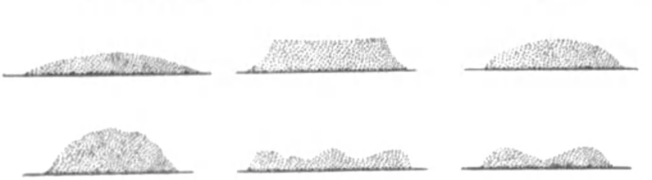


Fig. 34. Colonies of a different structure (cross-sectional view)

divided into large (4-5 mm in diameter), intermediate (2-4 mm), small (1-2 mm) and dwarf (less than 1 mm).

The colonies differ in their consistency, density and colour. They may be transparent and opaque, coloured and colourless, moist, dry and slimy.

In liquid nutrient media microbes grow producing a diffuse suspension, film or precipitate visible to the naked eye.

The growth of bacteria in the laboratory is carried out in test tubes, Petri dishes and flasks.

In institutes for production of vaccines and sera the cultivation of aerobes is carried out by deep stab methods. This method permits a more rational use of the nutrient substrate, and a large microbial mass can be obtained. The cultures are grown in reactors of a volume up to 1,000 litres (Fig. 35). Aeration is produced by pass-

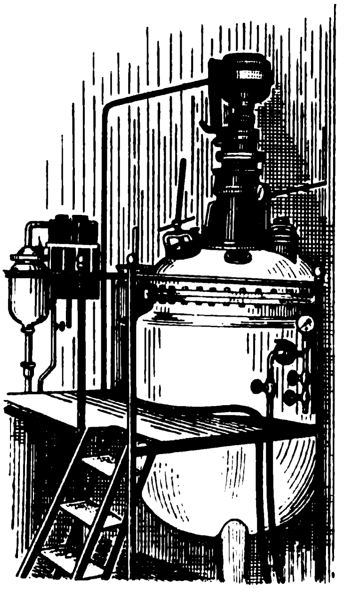


Fig. 35. General view of reactor

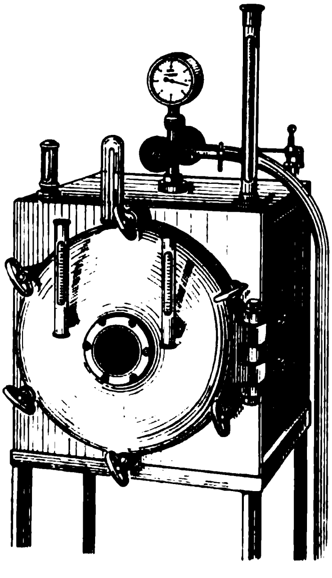


Fig. 36. Anaerostat

ing a stream of air through the medium. The method of aeration is used in laboratory investigations to encourage rapid growth of bacteria and to study some processes of metabolism.

Reproduction in microbes takes place more intensively in a flowing nutrient medium which is constantly being renewed. For this purpose a spare tank with nutrient medium is installed, from which the medium enters the cultivator and is carefully mixed with the culture. After this the excess of cultural fluid together

with the suspended bacterial cells flows out. When the rate of flow of cells from the cultivator is equal to the rate of reproduction, the number of the microbial population remains constant.

In usual laboratory conditions anaerobes develop in stationary (Fig. 36) or portable anaerobic jars containing rarified air up to 1-8 mm or in vacuum desiccators.

For successfully cultivating anaerobes it is necessary to seed a large amount of material into the nutrient medium. The nutrient medium should have a certain viscosity which is attained by adding 0.2 per cent agar. The air is removed by boiling prior to seeding, and to inhibit the subsequent entry of air, the medium is covered with a layer of oil 0.5-1 cm thick. Anaerobiosis is obtained by the adsorption of oxygen on porous substances (pumice, cotton wool, coal) and by adding reducing substances (carbohydrates, peptone, cysteine, pieces of liver, spleen, kidneys, brain, etc.). After seeding, the test tubes are filled up with liquid vaseline. Growth of the anaerobes usually is carried out on a Kitt-Tarozzi medium consisting of broth, 0.5 per cent glucose and pieces of animal organs (liver) or minced meat, and also in deep agar cultures or in special tubes filled with meat-peptone agar and sealed at the ends (Vignalou-Veillon).

One of the simplest means of cultivating microbes is by Fortner's method. One half of a Petri dish containing solid nutrient medium is seeded with a known aerobic microbe and the second half with the material under test containing the assumed anaerobes. The edges of the dish are covered with a special sealing wax, and then the dishes are placed in a thermostat. First the aerobes begin to grow until all of the oxygen is exhausted, after which the anaerobes begin to grow.

For the cultivation of pathogenic spirochaetes and protozoa special nutrient media are employed containing native proteins (serum, ascitic fluid, blood) and pieces of fresh organs and tissues (rabbit kidney, brain tissue, chicken embryo tissue).

Cultivation of rickettsiae. Rickettsiae are intracellular parasites, living in artificial nutrient media containing outliving tissues. Rickettsiae reproduce in tissues with a decreased metabolism.

The cultivation of rickettsiae in chick embryos according to the method of Cox. The material containing rickettsiae is injected into the yolk sac (see p. 104). Infected eggs are placed in the incubator at 36-37°C for 6-7 days.

To obtain a large amount of rickettsiae, white mice are infected intranasally, and the required amount of rickettsiae accumulates in the lungs.

The growth of rickettsiae by the method of Weigl and Mossing. Clothes lice are infected with a suspension of rickettsiae by introducing a special capillary tube into the gut through the anal opening of the lice. A. Pshenichnov and B. Reicher devised a method of

cultivating rickettsiae on the larvae of lice, which were fed defibrinated blood through a dead dermal membrane. G. Sneider and C. Wippler used the skin of a chick as an epidermal membrane.

These methods are used to prepare typhus fever vaccine and antigens for the serological diagnosis of typhus fever and other rickettsioses.

Cultivation of viruses. Viruses are not capable of binary fission, and multiple division or budding has not been observed among this group of organisms. When the virus penetrates the cell, its nucleic acid is freed of its protein and protein membrane. The protein components of the virus are reformed in the infected protoplast by the information received through the viral nucleic acid. Meanwhile, the "building up" or composition of the component parts of the virus from the protein molecules formed in the protoplast takes place.

Viruses in contrast to bacteria do not reproduce in common nutrient media. For their growth noninoculated, primarily inoculated and inoculated tissue cultures are employed. Over the years the methods of cultivation of viruses have undergone marked changes.

The introduction of tissue cultures into virological practice has been very important and has allowed the rapid development of virology.

The first successful transplantation was carried out in 1885 by V. Roux who for a number of days maintained the cells of a chick embryo in a warm salt solution. In 1887 G. Arnold preserved frog leucocytes in a warm salt solution. In 1903 G. Jolie observed the tolerance and division in vitro of salamander leucocytes in a hanging drop for almost a month. S. Beebe and G. Young in 1906 described real tissue cultivation. They obtained a tissue culture of infectious dog lymphosarcoma. In 1907 R. Harrison succeeded in inducing a multiplication of the cells of a frog embryo in aseptic conditions in a clump of lymph. He also introduced into practice cultivation on glass. A. Carrel devised a method by means of which it was possible to keep in vitro tissue cultures capable of active multiplication over a long period. A. Carrel initiated a new trend which introduced the method of long and constant cultivation of rapidly growing and dividing cells.

In 1928 H. Maitland and M. Maitland worked out a very simple method (the suspension of pieces of tissue in a liquid medium) of tissue culture for the multiplication of viruses. J. Enders and colleagues proved that tropism in viruses is not absolute. They grew in vitro the poliomyelitis virus in a tissue culture lacking nerve cells.

The technique of tissue culture began to develop and be perfected especially rapidly in the last ten years. This has been due to the use of electron microscopy methods and a more profound study of the physiology, biochemistry and genetics of tissue cultures.

At present strains of tissue cultures with specific characteristics have been received. The method of obtaining tissue cultures, their

classification and methods of application are described in detail in special guides.

Recently data have appeared on the possibility of virus multiplication outside of tissue cells. Thus, for example, it was observed that the infection factor increased in homogenates of disintegrated cells, incubated with the infectious RNA of the polio virus. The maximum accumulation of infected material was noted within three hours after infection, while the development of the infection was accompanied by an intense synthesis of nucleic acids and protein, and an increase in the activity of the cellular polynucleotide phosphorylase. Possibly, for the development of viruses the presence of live material is sufficient and it need not necessarily be in cellular form.

In 1962 the synthesis of tobacco mosaic virus was made possible in a medium without live cells, but consisting of nucleotides and energy systems. Within 30 minutes in this medium more than 100 million new virus particles were observed.

In recent years a method has been devised for obtaining tissue cultures from the embryos of humans, monkeys, guinea pigs and other animals.

The tissue cultures of SHS (simian heart sinomolgus), kidney tissue of monkeys, guinea pigs, tissue cultures of tonsils, placenta, etc., have been successfully used.

A widespread application was given to cells of tumours (HeLa, Hep-2, Detroit-6, etc.). The HeLa tissue culture (see Fig. 37) is made up of tissue cells from a woman Helen L. who died of cancer. These cells are able to grow rapidly in the corresponding physiological solutions. HeLa cultures are used in virological laboratories the world over for cultivating viruses of poliomyelitis, tick-borne encephalitis, etc.

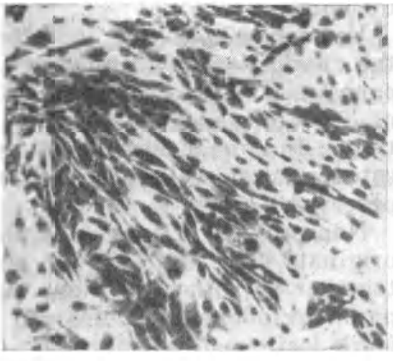


Fig. 37. HeLa tissue culture

Single layer tissue cultures have a great advantage over other methods of virus cultivation. They grow rapidly, and make it easy to observe the cytopathogenic effect. There are many indications as to the extensive infection of tissue cultures by various representatives of the genus *Mycoplasma*, which requires a strict control of the purity of these cultures.

For the growth of cells of tissue cultures the synthetic medium No. 199 is widely used. It is composed of more than 60 ingredients (amino acids, vitamins, glucose, purine, pentose, salts, etc.). It is

capable of maintaining the growth of tissues for 3-4 weeks. It is prepared commercially as a concentrate. Before using, the medium is diluted 10 times with thrice distilled water and 10-20 per cent of human or bovine serum are added.

Besides the synthetic medium a semisynthetic medium is used which contains amino acids, vitamins, a salt solution and 5-10 per cent of human blood serum.

The tissue cells are treated with trypsin to free them from the main bulk of the connective tissue. They are carefully washed with a phosphate buffer solution, and then a nutrient medium is added after which the solution is poured into flat-bottomed flasks which are placed in an incubation chamber. In 3-7 days the cells begin to grow. The monolayer culture is washed with a phosphate buffer solution, and is streaked with material containing the virus. It is grown from 10 to 20 days depending on the species of virus. The liquid with the nutrient medium is poured from the flasks into test tubes, and cultivated again for a certain period of time. To reveal the cytopathogenic activity the culture is examined under a special magnifying glass. When necessary the virus is studied by using serological reactions and biological tests on animals.

The cultivation of viruses in chick embryo. This method is used for the cultivation of more than 30 species of viruses and rickettsiae.

The material containing the virus is injected into the chick embryo (Fig. 38) in different ways: into the amnion, allantois, yolk sac, intravenously, intracerebrally, into the eyes, and intraperitoneally.

Various comparative investigations of tissues and the fluids of the chorioallantoic membrane were carried out to establish specific changes. Thus, for example, uninfected chorioallantois incubated at 37°C for 48-96 hours keeps its normal transparency and elasticity. In normal membranes disturbances of the integrity of the ectodermal epithelium and accumulations of inflamed cells are not observed.

Nonspecific reactions and affections of the chorioallantois may originate as a result of the action of a foreign tissue introduced together with the infectious material. During this, spotted transparent ectodermal papules and small irregular ulcerations develop. Dark foci, small haemorrhages and moderate swellings occur along the blood vessels. Nonspecific changes usually localize in the area where the infectious material was incorporated. Secondary foci do not occur.

Specific lesions in 10-11-day-old membranes develop in the form of a diffuse turbidity and a swelling with abundant ulcers, areas of necrosis and haemorrhages (Fig. 39). In 12-14-day-old membranes infected with smallpox virus or vaccinia virus quite frequently foci of pox-like lesions occur. The specific active infec-

tious process is characterized by the presence of a diffuse turbidity, swellings, haemorrhages, focal thickenings or pustules, boils, ulcers, areas of necrosis and accumulation of the inflammatory exudate on the surface and inside the membrane, and the development of secondary infections and death of the embryo.

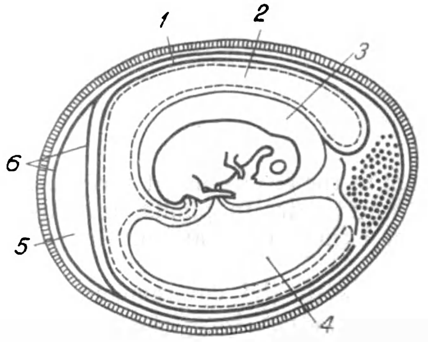


Fig. 38. Chick embryo

1—chorioallantois; 2—allantoic cavity; 3—amnitic cavity; 4—yolk sac; 5—air chamber; 6—outer-shell membrane

Fig. 39. Specific changes in the chorioallantois of a 12-day-old chick embryo infected with the vaccinia virus

When investigated under the microscope, areas of necrosis, thrombosis, capillary destruction, hyperplasia, hypertrophy, focal cellular proliferations, and the presence of inclusions and other changes in the cytoplasm and nucleus are found to have occurred.

The specificity of the infectious process is regulated by infecting laboratory animals susceptible to this virus, and also by a specific prevention of the infection with immune serum injected with the infectious material into the chorioallantoic membrane.

THE DISTRIBUTION OF MICROBES IN NATURE

Microbes are distributed everywhere in the environment surrounding us. They are found in the soil, water, air, in plants, animals, food products, various utensils, in the human body and on the surface of the human body.

The relationship of microorganisms with the environment has been named *ecology* (Gr. *oikos*—home, native land, *logos*—idea, science). This is an adaptive relationship. Microorganisms have a remarkable ability to adapt themselves to certain environmental conditions.

SOIL MICROFLORA

Soil science was founded by V. Dokuchaev, P. Kostychev, S. Vinogradsky, V. Williams, etc. Soil fertility depends not only on the presence of inorganic and organic substances, but also on the presence of various species of microorganisms which influence the qualitative composition of the soil. Due to nutrients and moisture in the soil the number of microbes in 1 g of soil reaches a colossal number—from 200 million bacteria in clayey soil to 5 thousand million in black soil. One gram of the ploughed layer of soil contains 1-10 thousand million bacteria.

Soil microflora consists of algae, nitrifying, nitrogen-fixing, denitrifying, cellulose-splitting and sulphur bacteria, pigmented microbes, fungi, protozoa, etc.

The extent to which the soil is contaminated with microbes depends on its nature and chemical composition (Table 6).

The greatest amount of microbes (1,000,000 per cu cm) is found in the top layer of soil at a depth of 5-15 cm. In deeper layers (1.5-5 m) individual microbes are found. However, they have been discovered at a depth of 17.5 m in coal, oil and artesian water.

It has been calculated that in the ploughed layer of cultivated soil over an area of 1 hectare there may be from 5 to 6 tons of microbial mass.

Table 6

Total Amount of Microbes in Different Soils According to the Direct Counting Method

Kind of soil	Number of microbes per 1 g	Number of spores in 1 g
Clayey podsol	801,800,000	4,000
Forest soil	1,219,000,000	12,000
Chernozem	4,771,000,000	100,000-180,000
Sandy soil	2,854,000,000	200,000-400,000
Light soil	2,661,100,000	700,000-
Loose sand	904,000,000	600,000-1,200,000
Gray soil	896,000,000	750,000-1,500,000

The number of microorganisms in the soil depends on the extent of contamination with faeces and urine, and also on the nature of treating and fertilizing the soil. For example, ploughed soil contains 2.5 times more microbes than forest soil.

Saprophytic spores (*B. cereus*, *B. megaterium*, etc.) survive for long periods in the soil.

Pathogenic bacteria which do not produce spores due to lack of essential nutrients, and also as a result of the lethal activity of light, drying, antagonistic microbes and phages do not live long in the soil (from a few days to a few months) [Table 7].

Table 7

Survival Period of Pathogenic Bacteria in the Soil

Species of bacteria	Average period in weeks	Maximal period in months
Salmonella typhosa	2-3	12
Shigella	1.5-5	9
Vibrio cholerae	1-2	4
Mycobacterium tuberculosis	13	7
Brucella	0.5-3	2
Pasteurella pestis	0.5	1
Pasteurella tularensis	1.5	2.5

Usually the soil is an unfavourable habitat for most pathogenic species of bacteria, rickettsiae, viruses, fungi, and protozoa. The survival period of some pathogenic bacteria is shown in Table 7. However, the soil as a factor of transmitting a number of causative agents of infectious diseases is quite a complex substrate. Thus, for example, anthrax bacilli after falling on the soil produce spores which can remain viable for many years. In favourable conditions (in dark brown soil and chernozem) they pass through the whole

cycle of development: during the summer months the spores germinate into the vegetative forms and then this cycle is repeated.

As is known, the spores of clostridia causing tetanus, gas gangrene and botulism, and of many soil microbes survive for long periods in the soil. The soil is the habitat for various animals (rodents) which are parasitized by the carriers of the causative agents of plague, tularaemia, the viruses of mosquito fever, haemorrhagic fever, encephalitis, agricultural leishmaniasis, etc. The cysts of intestinal protozoa (amoeba, balantidium, etc.) spend a certain stage in the soil. The soil plays an important role in transmitting worm invasions (ascarids, hookworms, nematode worms, etc.). Some fungi live in the soil. Entering the body they cause fusotoxicosis, ergotism, aspergillosis, penicilliosis, mucormycosis, etc.

Taking into consideration the definite epidemiological role played by the soil in spreading some infectious diseases of animals and man, sanitary-epidemiologic practice involves measures directed at protecting the soil from pollution and infection with pathogenic species of microorganisms.

S. Vinogradsky, V. Omeliansky, N. Kholodny, et al, devised a method of investigating soil microbes and used the results obtained in agriculture.

A valuable index of the sanitary condition of the soil is the discovery of the colibacillus and related bacteria, also enterococci, and *Clostridium perfringens*. The presence of the latter indicates an earlier faecal contamination.

MICROFLORA OF THE WATER

Pseudomonas fluorescens, *Micrococcus candidus*, *Micrococcus agilis*, etc., are among the specific aquatic aerobic microorganisms. Anaerobic bacteria are very rarely found in water.

The microflora of rivers depends on the degree of pollution and the quality of purification of sewage waters flowing into river beds. Thus, for example, in 1 ml of water in the Spree River 50,000,000 microbes (about 2.5 million in 1 drop of water) were found by the plating method, in tap water—from 2 to 50 microbes. By the method of direct counting on filters considerably more microorganisms were found.

Microorganisms are widespread in the waters of the seas and oceans. They have been found at different depths (3,700-9,000 m). In the Pacific Ocean unusual microorganisms (filamentous-clusterlike) were revealed with heads made up of round structures. It has been suggested that they are the most ancient microorganisms of our planet.

The degree of contamination of the water with organisms is expressed as saprobity which designates the total of all living matter

in water containing accumulations of animal and plant remains. Water is subdivided into three zones. *Polysaprobic* zone is strongly polluted water, poor in oxygen and rich in organic compounds. The number of bacteria in 1 ml reaches 1,000,000 and more. Colibacilli and anaerobic bacteria predominate which bring about the processes of putrefaction and fermentation. In the *mesosaprobic* zone (zone of moderate pollution) the mineralization of organic substances with intense oxidation and marked nitrification takes place. The number of bacteria in 1 ml of water amounts to hundreds of thousands, and there is a marked decrease in the number of colibacilli. The *oligosaprobic* zone is characteristic of pure water. The number of microbes is low, and in 1 ml there are a few tens or hundreds; this zone is devoid of the colibacillus.

Depending on the degree of pollution pathogenic bacteria can survive in reservoirs and for a certain time can remain viable. Thus, for example, in tap water, river or well water salmonellae of enteric fever can live from 2 days to 3 months, shigellae—5-9 days, leptospirae—from 7 to 150 days. The cholera vibrio lives in water for many months, the causative agent of tularaemia—from a few days to 3 months.

Tap water is considered clean if it contains a total amount of 100 microbes per ml, doubtful if there are 100-150 microbes, and polluted if 500 and more are present. In well water and in open reservoirs the amount of microbes in 1 ml should not exceed 1,000. Besides, the quality of the water is determined by the presence of *E. coli* and its variants.

The degree of faecal pollution of water is estimated by the colititre or coli-index. *The colititre* is the smallest amount of water in millilitres in which at least one *E. coli* bacillus is found. *The coli-index* is the number of individuals of *E. coli* found in 1 litre of water. Tap water is considered good if the colititre is within the limits of 300-500. Water is considered to be good quality if the coli-index is 2-3. Water in mine wells should have a colititre of not less than 100.

Due to the fact that enterococci are constant inhabitants only of the intestine in man and warm-blooded animals, and are highly resistant to temperature variations and other environmental factors, they are taken into account with the colititre and coli-index for the determination of the degree of faecal pollution of water, sewage waters, soil and other objects. At present new standards of enterococcus indices are being worked out.

Water is an important factor for the transmission of a number of infectious diseases (enteric fever, paratyphoids, cholera, dysentery, leptospiroses, etc.).

Due to the enormous sanitary-epidemiological role of water in relation to the intestinal group of diseases, it became necessary to

work out rapid indicator methods for revealing colibacillus and pathogenic bacteria in water.

These include the methods of luminescent microscopy for the investigation of water for the presence of pathogenic microbes and the determination of the increase of the titre of the phage. Upon the addition of specific phages to liquids containing a homologous microbe in 5-10 hours a considerable increase in the amount of phage particles can be observed.

For a more complete and profound study of the microflora of the soil and water *capillary microscopy* is used. The principle is that very thin capillary tubes are placed in the soil or water reservoirs after which their contents are exposed to microscopic investigations. This method reveals those species of microorganisms which do not grow in ordinary nutrient media, and which for many years were unknown to microbiologists.

MICROFLORA OF THE AIR

The composition of the microbes of the air is quite variable. It depends on many factors: on the extent to which air is contaminated with mineral and organic suspensions, on the temperature, rainfall, locality, humidity, and other factors. The more dust, smoke and soot in the air, the greater the number of microbes. Each particle of dust or smoke is able to adsorb on its surface numerous microbes. Microbes are rarely found on the surfaces of mountains, in the seas of Arctic lands covered with snow, in oceans and in snow.

The microflora of the air consists of very different species which enter it from the soil, plants, and animal organisms. Pigmented saprophytic bacteria (micrococci, various sarcinae), cryptogams (hay bacillus, *B. cereus*, *B. megaterium*), actinomycetes, moulds, yeasts, etc., are often found in the air.

The number of microbes in the air varies from a few specimens to many tens of thousands per 1 cu m. Thus, for example, the air of the Arctic contains 2-3 microbes per 20 cu m. In industrial cities large numbers of bacteria are found per 1 ml of air. In the forests, especially coniferous forests, there are few microbes; the volatile plant substances, phytoncides, having bactericidal properties which cause a lethal effect.

According to the investigations of E. Mishustin, above Moscow at an altitude of 500 m in 1 cu m of air from 1,100 to 2,700 microbes were found, while at an altitude of 2,000 m there were only from 500 to 700. Microbes (sporulating and moulds) were found at an altitude of 20 km. One gram of dust contains up to 1 million bacteria. Pathogenic species of microbes (pyogenic cocci, tubercle bacilli, anthrax bacilli, bacteria of tularaemia, rickettsiae of Q-fever,

etc.) may be found in the surroundings of sick animals and humans, infected arthropods and insects, and in dust.

At present *Streptococcus viridans* serves as sanitary indices for the air of closed buildings, and haemolytic streptococci and pathogenic staphylococci are a direct epidemiological hazard.

Depending on the time of the year, the composition and the amount of microflora change. If the total amount of microbes in winter is accepted as 1, then in spring it will be 1.7, in summer —2 and in autumn—1.2.

The total amount of microbes in an operating room before operation should not exceed 500 per 1 cu m of air, and after the operation not more than 1,000. There should be no pathogenic staphylococci and streptococci in 250 litres of air. In operating rooms of maternity hospitals before work the number of saprophytic microflora colonies isolated from the air by precipitating microbes on meat-peptone agar within 30 minutes should not exceed 20. In 1 gram of dust in hospitals, there are up to 200,000 pyogenic (haemolytic) streptococci.

The number of microbes in factories and homes is associated closely with the sanitary hygienic conditions of the building. In overcrowding, poor ventilation and natural lighting and if the premises are not properly cleaned, the number of microbes increases. Dry cleaning processes, infrequent floor washing, the use of dirty rags and brushes, and drying them in the same room make the conditions favourable for the accumulation of microbes in air. The causative agents of influenza, measles, scarlet fever, diphtheria, whooping cough, tonsillitis, acute catarrhs of the respiratory tract, tuberculosis, smallpox, pneumonic plague and other diseases can be transmitted through the air together with droplets of mucus and sputum during sneezing, coughing and talking.

Microbes can be spread by air currents, by aerial dust and aerial droplets. K. Flugge, P. Laschenkov and others proved that during sneezing, coughing and talking, a sick person can expel pathogenic bacteria together with droplets of mucus and sputum into the surrounding environment within a radius of 1-1.5 m or more.

Microorganisms contained in air can remain in three phases of the bacterial *aerosol*—droplet, droplet-nuclei and dust. An aerosol is the physical system of solid or liquid particles suspended in a gaseous medium.

On the average a person breathes about 12,000-14,000 litres of air daily, while 99.8 per cent of the microbes contained in air are held back in the respiratory tract. The bacterial aerosol produced naturally in the nasopharynx, during sneezing and coughing is thrown into the air—up to 60,000 droplets of different size. Among them almost 60 per cent consist of large droplets (100 mμ), 30 per cent—of average sized droplets (50 mμ) and 10 per cent—of small (5 mμ) droplets.

The greatest amount of bacteria is discharged during sneezing, less—during coughing, and still less—during talking. With each sneeze a man expels from 10,000 to 1,000,000 droplets. In one cough from 10 to 1,000 droplets containing bacteria are discharged into the environment, and when a person utters 10-20 words—up to 80 droplets are expelled. The nature of the bacterial aerosol depends on the viscosity of the secretion excreted from the respiratory tract. A liquid secretion is dispersed into more minute droplets more easily than a viscous one. Near the person expelling the bacteria a more concentrated aerosol of bacterial droplets from 1 to 2,000μ in size is produced. Most of the droplets are from 2 to 100μ in size. Large droplets from 100 to 2,000μ in size are thrown out to a distance of 2-3 m and more and quickly settle on the ground. Small drops of the bacterial aerosol (1-10μ) can remain in a suspended condition for a long time (for hours or days).

The air is an unfavourable medium for microbes. The absence of nutrient substances, the presence of moisture, optimal temperature, the lethal activity of sunlight and desiccation do not create conditions for keeping microbes viable and most of them perish. However, the relatively short period during which the microbes are in air is quite enough to bring about the transmission of pathogenic bacteria and viruses from sick to healthy persons, and to cause extensive epidemics of diseases such as influenza.

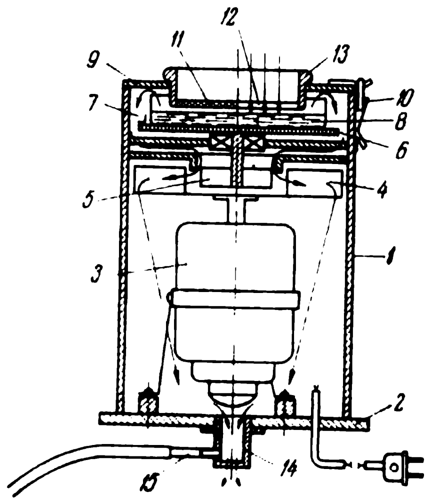


Fig. 40. Krotov's apparatus

1—cylindrical housing; 2—housing base; 3—electric motor; 4—fan; 5—eight-blade impeller; 6—disk; 7—springs; 8—Petri dish; 9—lid of apparatus; 10—clasp locks; 11—plexiglass disks; 12—V-shaped slot; 13—split ring; 14—pipe connection with diaphragm; 15—outlet pipe

For the purpose of prophylaxis various methods are used in protecting humans from infection via air-borne dust. Thus, the sputum of tuberculosis patients is burned or decontaminated, the room is frequently ventilated, and cleaned by mopping, the streets are sprayed, drainage and absorbers are used, and masks are used during sorting of wool and rags, etc. The air of operating rooms, isolating rooms, wards, and bacteriological laboratories is decontaminated by ultraviolet radiation (mercury-quartz, uviol lamps, etc.).

The laboratory investigation of air is carried out to determine the qualitative and

quantitative composition of its microflora. This is achieved by using simple and complex methods. For a more accurate investigation of microbial contents of the air special apparatus is used, e.g., Rechmensky bacterial absorber, Krotov's apparatus (Fig. 40), etc.

At present rapid methods for the indication of microbes in the environment are being devised which will allow quick determinations of the presence of microorganisms in the soil, the water and air.

NORMAL MICROFLORA OF THE HUMAN BODY

Human microflora is the result of a mutual adaptation of micro- and macroorganisms in the process of evolution. Most bacteria of the normal and constant microflora of the human body have adapted themselves to life in certain parts of the body. Besides, there are some microbes which make up a temporary (casual) microflora.

With the development of virology and the improvement of virological technique, our concepts on the microflora of the human body were increased. It has been established that not only the open cavities, but the tissues of the human organism are inhabited by numerous viruses which are excreted into the environment with milk, saliva, sputum, perspiration, urine and faeces.

Microflora of the skin. Staphylococci, streptococci, moulds and yeasts, diphtheroids and also certain pathogenic and conditionally pathogenic bacteria live on the surface of the skin. They receive their nutrition from the secretions of the sebaceous and sweat glands, dead cells and waste products.

P. Remlinger established that the total number of microbes on the skin of one person varies from 85,000,000 to 1,212,000,000.

When the human body comes into contact with the soil, the clothes and skin are seeded with spores of different species of microbes (organisms responsible for tetanus, gas gangrene, etc.).

Most frequently the exposed parts of the human body are infected, e.g., the hands, on the surface of which are found colibacilli, staphylococci, streptococci, enterococci, moulds, yeasts, fungi imperfecti, and spores of aerobic and anaerobic bacilli.

Violation of sanitary hygienic conditions and normal conditions of the work and life of people often are the cause of pustular and fungal infections of the skin and of gastrointestinal diseases.

Microbes of the mouth cavity. In the mouth cavity (see Fig. 117,*2*) there are more than 100 species of microbes. There are the natural inhabitants (acidophilic bacillus, *Treponema microdentium*, diplococci, streptococci, micrococci, *Entamoeba gingivalis*, etc.). Besides, in the mouth cavity there are foreign microbes or those which have been carried in from the environment together with food, water and air.

Pathogenic and conditionally pathogenic microbes (staphylococci, streptococci, pneumococci, diphtheria bacilli, diphtheroids, *Borrelia* organisms, spindle-shaped bacteria), protozoa (amoebae and trichomonads) are found on the mucous membrane of the mouth.

The mouth cavity is a favourable medium for many microbes; it has an optimal temperature, a sufficient amount of food substances and has a weakly alkaline reaction.

The greatest amount of microbes can be found at the necks of the teeth and in the spaces between teeth. Streptococci and diplococci are found on the tonsils. There are many microbes in other parts of the mouth cavity, which are inaccessible to the bathing action of saliva and the action of lysozyme (an antibiotic found in the saliva, lacrimal fluid and sputum). The presence of carious teeth is a condition for increasing the microflora in the mouth cavity, for the appearance of decaying processes and unpleasant odours.

The microflora of the gastrointestinal tract. When the stomach functions normally, it is almost devoid of microflora due to the marked bactericidal properties of gastric juice.

The gastric juice is considered to be a reliable defense barrier against the penetration of pathogenic and conditionally pathogenic microbes into the intestine. However, the degree of acidity of the gastric juice is not always constant. It varies according to the character of the food and the amount of water consumed. Together with food, lactic acid bacteria, *Sarcina ventriculi*, hay bacillus, yeasts, etc., enter the stomach from the mouth. In some cases, dysentery, enteric fever and paratyphoid bacilli and other pathogenic microbes are capable of penetrating into the stomach and then the intestine.

Enterococci, fungi and various other microbes are relatively rarely found in the duodenum. There are few microbes in the small intestine. Enterococci are found more often than others.

In the large intestine there are large amounts of microorganisms. Almost one-third of the dry weight of the faeces of certain animal species is made up of microbes. Daily, an adult human excretes about 17 million billion microorganisms with the excrements.

The intestinal microflora undergoes essential changes with the age of man. The intestinal tract of the newly-born in the first hours of life is sterile. During the first days it becomes inhabited by temporary microflora from the environment, mainly from breast milk. Later on, in the intestine of the newly-born a specific bacterial flora is established consisting of lactic acid bacteria (biphidobacteria, acidophilic bacillus), which is retained during the year. It has antagonistic properties in relation to many microbes capable of causing intestinal disorders in breast-fed children, and remains during the whole period of breast feeding. However, on the 3-5th day of life in the intestine of breast-fed children *E. coli* and enterococci can be found, the amount of which sharply increases with

the change to mixed feeding. After breast feeding is stopped the microflora of the child's intestine is completely replaced by a microflora typical of adults (*E. coli*, *Clostridium perfringens*, *Clostridium sporogenes*, *Streptococcus faecalis*, *Proteus vulgaris*, etc.).

At present it has been established that such a constant inhabitant of the intestine of man as *Clostridium perfringens* is capable of secreting digestive enzymes. The colibacillus and other species of microbes in the intestine produce the vitamins essential for the human body (B_1, B_2, B_{12}, K). Microbe antagonists (acidophilic, *Bacillus bulgaricus*, etc.) are beneficial to the organism as they hinder the development of pathogenic bacteria which, together with infected food and water, may enter the intestine.

The pathogenic serotypes of *E. coli* which are capable of causing severe diseases (colienteritis) mostly in children have been found to be present in the human intestine together with the nonpathogenic species.

Anaerobic bacteria which do not produce spores, the so-called *bacteroids*, inhabit mainly the lower part of the large intestine in humans. They are found during acute appendicitis, postpartum infection, pulmonary abscesses, septicaemias of different aetiology, postoperative infectious complications in the peritoneal cavity, inflammatory processes of the gastrointestinal tract, respiratory tract and on the skin.

E. Metchnikoff considered some species of intestinal bacteria to be harmful, causing chronic intoxications. He suggested the method of combating them by introducing lactic acid bacilli (*Bacillus bulgaricus*) bearing antagonistic properties into the intestine. Besides, E. Metchnikoff recommended a diet of vegetables and fruit, rich in sugar, and considered it advisable to build one's life according to the principles of *orthobiosis* (normal work, healthy relaxation, hygienic conditions and prophylaxis of diseases).

Enteroviruses live in large quantities in the intestine. They may be found for a long time in healthy persons without causing diseases. In unfavourable conditions associated with some species of bacteria they cause the most varied clinical forms of disease.

Microflora of the respiratory tract. People breathe in a large number of dust particles and adsorbed microorganisms. Experimentally, it has been established that the amount of microbes in inspired air is 200-500 times greater than in expired air. Most of them are trapped in the nasal cavity and only a small amount enters the bronchi. The pulmonary alveoli and the terminal branches of bronchi are usually sterile. The upper respiratory tract (nasopharynx, pharynx) contains relatively constant species (*Staphylococcus albus*, streptococci, pneumococci, diphtheroids, *Gaffkya tetragena*, etc.).

If the defense mechanisms of the body are weakened as a result of cooling, starvation, vitamin deficiency or traumas, the con-

stant inhabitants of the respiratory tract become capable of causing different diseases (acute catarrhs of the respiratory tract, tonsillitis, pneumonia, bronchitis, etc.).

The nasal cavity contains a small amount of microbes. The mucous membrane of the nose produces mucin and lysozyme which have a bactericidal action. However, in spite of this, the nasal cavity has a relatively constant microflora (haemolytic or "nasal micrococcus", diphtheroids, non-haemolytic staphylococci, haemolytic staphylococci, pneumococci, saprophytic Gram-negative diplococci, capsular Gram-negative bacteria, haemoglobinophilic bacteria of influenza, *Proteus*, etc.). In 1 mg of nasal mucus L. Perets found about 1,800 microbes. In the respiratory tract, besides the bacterial microflora, many viruses, in particular, adenoviruses, can remain viable for long periods without causing pathological processes. It is possible that some viruses are conditionally pathogenic, and more than likely that they are saprophytes.

Microflora of the vagina. In the first 2 days after birth the child's vagina is sterile. Sometimes it contains a small amount of Gram-positive bacteria and cocci. After 2-5 days of life the coccal microflora becomes fixed and remains constant until puberty, when it is replaced by Döderlein's lactic acid bacilli.

During the menstrual cycle the contents of the vagina become alkaline which is favourable for the development of coccal microflora. During sexual life the microflora of the vagina changes, and many microbes appear which are introduced from outside.

The microflora of the vagina undergoes profound changes during gynaecological diseases (endometritis, metritis, ovaritis, etc.), and after abortions.

The vaginal contents of the healthy woman have a relatively high concentration of sugar and glycogen, and a low content of the diastatic enzyme and proteins. The pH is 4.7 during which all other microbes, except for Döderlein's lactic acid bacilli, cannot develop.

As has been established, the acid medium of the vagina depends on the presence of glycogen which under the influence of vaginal bacteria is transformed into mono- and disaccharides and then into lactic acid. The amount of glycogen depends on the function of the ovaries and the condition of the whole body.

Vaginal bacteria have antagonistic properties, and because of this normal microflora should be protected and should not be exposed to the harmful effect of medicines (antibiotics, sulphonamide preparations, rivanol, osarsol, potassium permanganate, etc.) to which Döderlein's lactic acid bacilli are more sensitive than the bacteria against which these substances are employed.

Microflora of the urinary tract. In men in the anterior part of the urethra there are *Staphylococci us albus*, diphtheroids and Gram-negative nonpathogenic bacteria. *Mycobacterium smegmatis* and my-

coplasmas are found on the external parts of the genitalia, and also in the urine of men and women.

The female urethra is usually sterile, in some cases it contains a small amount of nonpathogenic cocci.

The bacteria of the mucous membranes of the eyes include *Staphylococcus albus*, *Corynebacterium xerosis*, mycoplasmas, etc. When the organism is weakened or during visual disturbances and vitamin deficiency, the normal inhabitants of the mucous membranes may become relatively pathogenic and may cause different diseases of the mucous membrane, such as conjunctivitis, blepharitis and other suppurative processes.

The normal microflora is not constant but depends on the age, nutrition and general condition of the macroorganism. The microflora of the human body undergoes profound changes, especially during various diseases.

Disturbances in the species composition of the normal microflora occurring under the influence of infectious and somatic diseases, and long-term and irrational use of antibiotics bring about the state of *disbacteriosis.* This is characterized by disturbances in the assimilation of products of digestion, by changes in the enzymatic processes, and by the cleavage of ready-made physiological secretions. The territorial deviations of microflora cause a whole series of complications: intestinal dyspepsia, toxinfections, suppurative processes, catarrhs of the respiratory tract, pneumonia, candidiasis, etc.

THE ROLE OF MICROBES IN THE CYCLE OF SUBSTANCES

Chemical substances from which plant and animal organisms are composed undergo a continuous cycle which is of considerable significance. The transformation of biologically active chemical elements on the surface of the earth is carried out by the biochemical activity of microorganisms.

In the process of combustion, respiration and fermentation about 450 thousand million tons of organic compounds are oxidized every year (attended by the intake of oxygen) and the same amount is reduced in the process of photosynthesis (attended by the intake of CO_2 and excretion of O_2).

NITROGEN CYCLE

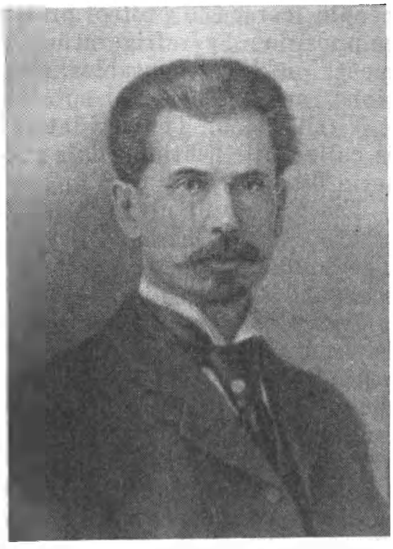
The study of the problems of the nitrogen cycle is one of the greatest achievements in microbiology in the last 75 years. Russian science deserves great merit in this field (S. Vinogradsky, V. Omeliansky, D. Pryanishnikov, et al).

Nitrogen makes up 78.11 per cent of the volume or 75.50 per cent of the weight of the atmosphere. Above each cubic metre of the earth is a layer of air containing 8 tons of nitrogen.

The gaseous free nitrogen is assimilated by green plants, animals and man. Bound nitrogen is not always fit for the nutrition of plants prior to its transformation into the salts of nitrous and nitric acids.

The putrefaction of proteins is the process of their decomposition to amino acids which, after deamination, produce phenol, cresol, indole, skatole, volatile fatty acids, methyl and ethyl mercaptans, hydrogen sulphide, ammonia and other substances. It is performed by the enzymatic activity of various species of aerobes (*Proteus vulgaris*, *B. cereus*, *Pseudomonas fluorescens*, hay bacillus, moulds) and anaerobes (*Clostridium sporogenes*, etc.). This process takes place in stages in conjunction with many species of microorganisms.

The cleaning of the surface of the soil from the cadavers of plants and animals takes place under the influence of putrefying microbes which mineralize organic substances to simple compounds (H_2O, CO_2, NH_3, $Mg(NO_3)_2$, $MgSO_4$, etc.).



S. N. Vinogradsky (1856-1953)

The degree to which proteins are decomposed under the influence of microbial enzymes is not the same. Deep cleavages are caused by *Proteus vulgaris*, *B. subtilis* and other sporulating aerobes, slight cleavages—by the cryptogamic anaerobes, *Pseudomonas fluorescens*, etc. The decay of proteins under anaerobic conditions is not as complete, and has been named *anaerobic putrefaction*. In aerobic conditions it is greater, and is known as *decay*. Putrefaction is inhibited at high and low temperatures. By means of low temperatures the dead bodies of mammoths have been preserved up to the present time in Siberia. Also in water at a depth of 4,000 m putrefaction does not take place. A human head found at the bottom of Tollund's turf swamp in Denmark is preserved in the Copenhagen Museum. Danish scientists consider it to have been there for about 2,000 years.

Ammonification. The cleavage of proteins with the production of NH_3 is called ammonification. The process of putrefaction of food remains begins in the digestive tract (*Clostridium sporogenes*, *Clostridium perfringens*, etc.) and is completed in the surrounding medium.

Ammonification of urea. The animal population of the world excretes more than 200,000 tons of urea—$CO(NH_2)_2$ (carbamide) daily, or almost 100,000 tons of urea nitrogen, the decomposition of which takes place under the influence of urobacteria and urosarcinae. These include *B. pasteuri*, *Sarcina ureae*, etc.

The reaction takes place as follows:

$$CO(NH_2)_2 + 2H_2O \longrightarrow (NH_4)_2CO_3$$
$$(NH_4)_2CO_3 \longrightarrow 2NH_3 + CO_2 + H_2O$$

Putrefaction is widely employed in sanitary and industrial practice (irrigation fields, sweating of hides).

Understanding the causes for the processes of putrefaction,

people learned to protect protein-containing food products from decomposition by refrigeration, salting, smoking and dry curing of meat, pickling vegetables, sugaring fruit, using supersonic vibrations, and radioactive substances, etc.

Nitrification. The oxidation of ammonium salts to nitric acid is called nitrification. This process is performed by nitrifying bacteria discovered by S. Vinogradsky.

The process of nitrification takes place in two phases.

The first phase: $2NH_3+3O_2 \rightarrow 2HNO_2+2H_2O+158$ Cal. The oxidation of ammonia is caused by *Nitrosomonas*, *Nitrosococcus* and *Nitrosospira*.

The second phase: $2HNO_2+O_2 \rightarrow 2HNO_3+43.2$ Cal. Nitrous acid is oxidized by *Nitrobacter*.

In the process of the historical development of the planet large deposits of saltpetre were produced by nitrification in Chile, Peru, India, Egypt, the USSR and other countries.

Denitrification. This is the process of the decomposition of nitrites and nitrates with the excretion of free nitrogen carried out under the influence of denitrifying bacteria: *Pseudomonas denitrificans*, colibacillus and other facultative anaerobic microbes harmful for agriculture.

Besides direct denitrification carried out by denitrifying bacteria, there is indirect denitrification which takes place as a result of chemical reactions between amines or amides and nitrous acid.

The fixation of atmospheric nitrogen is carried out by nodule bacteria in legume plants and by nitrogen-fixing bacteria, living free in the soil. Nodule bacteria include *Agrobacterium radiobacter* and nitrogen-fixing microorganisms include *Clostridium pasteurianum*, *Azotobacter*, actinomycetes, fungi, purple bacteria, blue-green algae and many others. The protoplasts of microbes devoid of their cellular structure are capable of fixing nitrogen. It has been established that in each hectare of ordinary soil every year 25-50 kg of nitrogen are fixed, and in cultivated soil and soil containing legume plants from 35 to 60 kg and 100 to 400 kg of nitrogen are fixed respectively. When harvesting cereal grains 50-70 kg are lost. The diagram of the nitrogen cycle in nature is shown in Fig. 41.

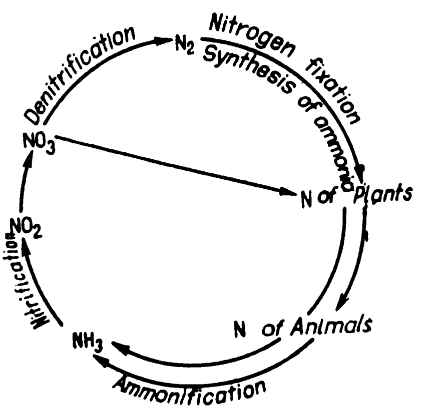


Fig. 41. Diagram of the nitrogen cycle in nature

On the basis of theoretical and experimental data different preparations have been obtained

for fertilizing the soil, which are extensively used in agriculture. These include nitragin (pure culture of nodule bacteria), azotobacterin (culture of *Azotobacter* in neutral turf or humus), phosphorobacterin, AMB preparation (autochronic microflora B) containing ammonifying, denitrifying, aerobic, cellulose-splitting, nitrifying and thiogenic bacteria.

CARBON CYCLE

It has been established that almost 50 per cent of living matter is composed of carbon. Green plants of the earth surface utilize more than 70 thousand million tons of carbon dioxide (20 thousand million tons of carbon) per year. The atmosphere contains 0.03 per cent of carbon dioxide in volume, which is of great significance in the life of plants and animals. If the supply of carbon dioxide were not replenished, it would have been exhausted within 40-50 years. This would have involved a sharp drop in temperature, making it impossible for plants to exist, and thus animal and human life would have ceased.

The replenishment of the necessary amount of carbon dioxide is carried out at the expense of volcanic eruptions and the decay of organic compounds. Since synthesis in plants considerably exceeds decomposition, a certain amount of carbon dioxide must come from other sources.

This deficiency cannot be replenished by the carbon dioxide which is given off by animal bodies in the process of respiration, but the lack of carbon dioxide is compensated by the biochemical activity of microorganisms.

The processes of the decomposition of nitrogen-free substances are provided for by fermentation (L. Pasteur), the processes of assimilation by *photosynthesis* in green plants (K. Timiryazev) and by *chemosynthesis*.

Photosynthesis is one of the most important biological processes, which takes place in the cells of green plants. With the help of the green pigment, chlorophyll, concentrated in intracellular structures, chloroplasts, the cells of green plants absorb light energy utilizing it for the synthesis of complex organic compounds from carbon dioxide and water.

Photosynthesis is an oxidation-reduction process in which water is decomposed liberating oxygen and releasing hydrogen for the reduction of carbon dioxide.

Photosynthesis takes place in stages. The first stage is characterized by the incorporation of the carbon dioxide into the carboxyl group by means of carboxylation:

$$CO_2 + RH \longrightarrow RCOOH$$

The second stage is accompanied by the reduction of the carboxyl group to the alcohol group.

$$R—COOH + 4H \longrightarrow R—CH_2OH + H_2O$$

In the third stage a molecule of carbon dioxide combines with the alcohol group.

$$R—CH_2OH + CO_2 \longrightarrow R—CHOH—COOH$$

Subsequently, this process continues until complex carbohydrates: fructose diphosphate, monosaccharides, saccharose and starch are produced.

Besides light energy for the production of glucose in the process of photosynthesis, chlorophyll needs carbon dioxide, water and certain minerals. The chemical equivalent of light energy is adenosine triphosphoric acid. Annually the plant world of our planet carries out the synthesis of complex carbohydrates with the absorption of 150,000,000,000 tons of carbon dioxide. About 90 per cent of this activity is carried out by microscopic algae found in water reservoirs.

Of interest are the investigations of unicellular photosynthetic algae (chlorella) which use sunlight quite effectively. They contain much protein, grow rapidly, and require a small amount of salts.

Fermentation. In the carbon cycle a great role is played by the decomposition of carbohydrates into more simple compounds. This process is known as fermentation, and is carried out by the fermentative activity of microorganisms. Depending on the product formed there are several types of fermentation (alcohol, lactic acid, butyric acid, butyl-acetic, etc.).

Alcoholic fermentation takes place under the influence of the enzymes of yeasts *Saccharomyces cerevisiae* (top yeasts), *Saccharomyces ellipsoides* (bottom wine yeasts), *Torula*, and mucor yeasts.

$$C_6H_{12}O_6 \longrightarrow 2CH_3CH_2OH + 2CO_2$$

Top yeasts cause the production of alcohol at a temperature of 20-28°C. This process is a rapid one with the formation of a foam on the surface of the fermenting liquid due to the production of carbon dioxide. Top yeasts are used in industrial alcohol production and in bakeries. Bottom yeasts are used in wine and beer making. Bottom fermentation is relatively slow and takes place at low temperatures (5-10°C).

In the process of alcoholic fermentation pyruvic acid and acetic aldehyde are produced from which ethyl alcohol is obtained by reduction with activated hydrogen.

As a result of the fermentative activity of acetic acid bacteria (*Acetobacter xilinum, Acetobacter aceti, Acetobacter pasteurianus*) alcohol is oxidized to acetic acid.

$$CH_3 - CH_2OH + O \longrightarrow CH_3 - COH + H_2O$$
$$CH_3 - COH + O \longrightarrow CH_3 - COOH$$

Lactic acid fermentation is caused by the fermentative activity of *Lactobacillus bulgaricus, Lactobacillus caucasicus, Streptococcus lactis*, etc. (the causative agents of typical lactic acid fermenta-

tion). The enzymes of lactic acid bacteria break down glucose with the production of lactic acid.

$$C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOOH$$

The *Serratia marcescens* bacillus, and bacteria of the genus *Proteus*, etc., bring about fermentation with the production of lactic, succinic, and acetic acids, ethyl alcohol, carbon dioxide and hydrogen.

The products of lactic acid fermentation include:

(1) *lactobacillin* (Metchnikoff's sour milk) prepared by fermenting pasteurized milk with cultures of *Lactobacillus bulgaricus*, *Streptococcus lactis*;

(2) *acidophilic milk* prepared by the fermentation of *Lactobacillus acidophilus* (Fig. 42);

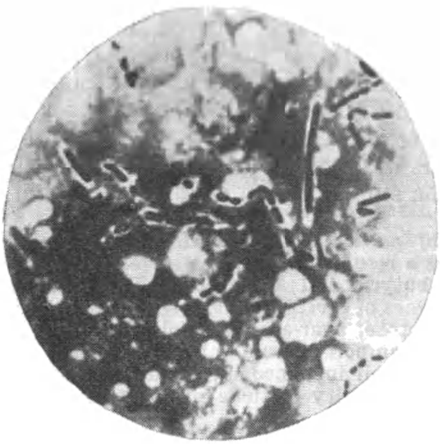


Fig. 42. *Lactobacillus acidophilus*

(3) *kefir*, a product of souring milk with kefir grains composed of milk proteins, lactic acid bacteria (*Lactobacillus acidophilus*), yeasts (*Torula ellipsoides*, *Torula kefir*), *Acetobacter aceti*, lactic acid streptococci and milk peptonizing bacteria from the genus *Proteus*;

(4) *koumiss*, a beverage from fresh mare's milk fermented by a special ferment containing *Lactobacillus bulgaricus*, *Lactobacillus caucasicus*, *Torula*.

Sour milk, matsoni, sour cream, cottage cheese, cheese, sour bread dough, bread ferment, bouza, silage, sauerkraut, pickled cucumbers and water melons, pickled apples, etc., are also products obtained mainly by lactic acid fermentation.

Butyric acid fermentation is brought about by the anaerobes *Clostridium butyricum*, *Clostridium pasteurianum* with the production of butyric acid

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 + 20 \text{ Cal}$$

As a source of carbon and energy for fermentation butyric acid microbes utilize alcohols (mannitol, glycerin) and organic acids (lactic and pyruvic). When these products are fermented, butyric acid, butyl alcohol, carbon dioxide and hydrogen are produced.

Butyric acid microbes are strict anaerobes. They develop well in conditions in which the amount of oxygen in the atmosphere does not exceed 20-30 mg per litre, and at a temperature of 30-40°C.

Butyric acid fermentation is widespread in nature. With its help plant remains in the soil, in the silts of reservoirs and other natural substrates which lack an access to oxygen, and also in the intestines of animals and man are broken down.

Under the influence of butyric acid microorganisms the fermentation of pectic substances, cellulose, and sometimes milk, cheese and other food products takes place with the production of an unpleasant odour and a bitter taste. Butyric acid fermentation may take place in pickled vegetables during a slow accumulation of lactic acid. It can cause the explosion of canned foods and the swelling of cheese. Butyric acid fermentation in some cases takes place in very moist flour giving it a bitter taste.

Complex ethers of butyric acid have a pleasant smell and are utilized as aromatic substances in confectionery and perfume making.

Fermentation of pectic substances. When flax is soaked in water, fermentation takes place as a result of the fermentative activity of *Clostridium pectinovorum* producing butyric and acetic acids. The fermentation of cellulose is caused by the enzymes of *Clostridium werneri*; other microbes cause the fermentation of starch, pentose, agar-agar, alcohols, fats.

Bacteria have an important role in treating rubber, cotton, silk, coffee, cocoa, tobacco. Under their influence important processes take place, which considerably change the quality of these products in the required manner.

THE CYCLE OF SULPHUR, PHOSPHORUS AND IRON

The oxidation of H_2S by sulphur bacteria takes place in the following way:

$$2H_2S + O \longrightarrow 2H_2O + S_2$$
$$S_2 + 2H_2O + 3O_2 \longrightarrow 2H_2SO_4$$

The salts of sulphuric acid (sulphates) are absorbed by plants.

Phosphoric acid with the alkaline earths of the soil produced poorly soluble salts which cannot be absorbed by the plants. *B. cereus* gives off carbon dioxide in the presence of which tricalcium phosphate is converted into a soluble dicalcium salt.

$$Ca_3(PO_4)_2 + 2CO_2 + 2H_2O \longrightarrow Ca_2H_2(PO_4)_2 + Ca(HCO_3)_2$$

The iron bacteria become saturated with ferric hydroxide and cause the accumulation of lake iron ore on the bottom of reservoirs.

The carbonate salts of ferrous oxide during oxidation are transformed into insoluble ferric hydroxides: $2FeCO_3 + 3H_2O + O \rightarrow Fe_2(OH)_6 + 2CO_2$

The investigations of N. Holodny and V. Butkevich established that the processes of reduction and solution of iron and manganese take place in deep

sediments while the processes of oxidation and deposition take place at the surface zone. When there is a lack of organic substances for the reduction of ferric oxide, oxide compounds are deposited on the bottom of water reservoirs which causes the accumulation of ferromanganese ores. In water pipes ferric oxide is deposited by the action of *Crenothrix polyspora* and causes clogging.

A whole series of putrifying, nitrifying, denitrifying, urolytic, nitrogen-fixing, cellulose-splitting and thianoacid microorganisms cause the corrosion of concrete and metals, producing ferric oxides.

It has been established that due to the fermentative processes of certain species of microorganisms oil and curing muds were produced.

Microorganisms are used as indicators. With their help the presence of certain processes or substances which cannot be revealed by chemical and analytical methods are established. These include oil mining, determination of the amount of fertilizer required by the soil, the determination of the exact amount of vitamins, amino acids and other substances. Microorganisms are also used as indicators in the hydrolytic processes in seas and oceans.

Microorganisms are able to synthesize different physiologically active substances, antibiotics, enzymes, and vitamins employed in medicine, veterinary practice and agriculture.

The synthetic capacity of microorganisms is extremely great. The total weight of the bacterial cytoplasm on Earth is considerably greater than the weight of animal cytoplasm. Therefore, the biochemical activity of microbes is no less important biologically than photosynthesis. The cessation of the existence of microbes would lead inevitably to the death of plants and animals.

THE INFLUENCE OF ENVIRONMENTAL FACTORS ON MICROBES

THE EFFECT OF PHYSICAL FACTORS

The effect of temperature. Microbes can withstand low temperatures fairly well. The cholera vibrio does not lose its viability at a temperature of —32°C. Some species of bacteria remain viable at a temperature of liquid air (—190°C) and of liquid hydrogen (—253°C). Diphtheria bacilli are able to withstand freezing for three months and enteric fever bacteria are able to live long in ice. Bacillus spores withstand a temperature of —253°C for 3 days. Many microorganisms remain viable at low temperatures, and viruses are especially resistant to low temperatures. Thus, for example, the virus of Japanese encephalitis in a 10 per cent brain suspension does not lose its pathogenicity at —70°C over a period of one year, the causative agents of influenza and trachoma—at —70°C for 6 months, and Coxsackie virus at —40°C for 1.5 years.

Low temperatures halt putrifying and fermentative processes. In sanitary hygienic practice ice, cellars, and refrigerators for the storage of food products are used according to this principle.

Only certain species of pathogenic bacteria are very sensitive to low temperatures (e.g., meningococcus, gonococcus, etc.). During short periods of cooling these species perish quite rapidly. This is taken into account in laboratory diagnosis, and materials under test for the presence of meningitis or gonorrhoea are conveyed to the laboratory protected from cold.

At low temperatures the processes of metabolism are inhibited, the bacteria die off as a result of ageing and starvation, and the cells are destroyed under the influence of the formation of ice crystals during freezing.

Alternate high and low temperatures are lethal to microbes. It has been established, for instance, that sudden freezing as well as sudden heating causes a decrease in the life activities of pathogenic microbes.

Most asporogenic bacteria perish at a temperature of 58-60°C within 30-60 minutes. Bacillus spores are more resistant than vegetative cells. They withstand boiling from a few minutes to 3 hours,

but perish under the effect of dry heat at 160-170°C in 1-1.5 hours. Heating at 120.6°C at 2 atm steam pressure kills them within 20-30 minutes.

Individual and specific variations in the resistance of microbes to high temperatures have different limits and a rather large temperature range.

The inhibition of the activity of catalase, oxydase, dehydrogenase, protein denaturation and an interruption of the osmotic barrier is the principle of the bactericidal action of high temperatures. High temperatures cause a rather rapid destruction of viruses, but some of them (viruses of epidemic hepatitis, poliomyelitis, etc.) are resistant to environmental factors. They remain viable long in water, in the faeces of sick people or carriers, and are resistant to heat at 60°C and to small concentrations of chlorine in water.

The effect of desiccation. Microorganisms have a different resistance to desiccation to which gonococci, meningococci, treponemas, leptospiras, haemoglobinophilic bacteria and phages are sensitive. On exposure to desiccation the cholera vibrio persists for 2 days, dysentery bacteria—for 7, plague—for 8, diphtheria—for 30, enteric fever—for 70, staphylococci and tubercle bacilli—for 90 days. The dry sputum of tuberculosis patients remains infectious for 10 months, the spores of anthrax bacillus remain viable for 10 years, and those of moulds for 20 years.

Desiccation is accompanied by dehydration of the cytoplasm and denaturation of bacterial proteins. Desiccation in a vacuum at a low temperature does not kill bacteria, rickettsiae or viruses. This method of preserving cultures is employed in the manufacture of stable long-storage, live vaccines against tuberculosis, plague, tularaemia, brucellosis, smallpox, influenza and other diseases.

Quick freezing of bacterial and viral suspensions at very low temperatures provokes conditions at which crystals do not form, and subsequent disruption of the microorganisms does not occur.

The effect of light. Some bacteria (purple) withstand the effect of light fairly well, while others are injured. Direct sunlight has the greatest bactericidal action.

Investigations have established that different kinds of light have a bactericidal or sterilizing effect. These include ultraviolet rays (electromagnetic waves with a wave length of 200-300 mμ), X-rays (electromagnetic rays with a wave length of 0.005-1 mμ), gamma-rays (short wave X-rays), beta-particles or cathode rays (high speed electrons), alpha-particles (high speed helium nuclei) and neutrons.

The experiments in which short waves were used for the disinfection of wards, infectious material, for the conservation of products, the preparation of vaccines, treating operating and maternity wards, etc., have demonstrated that they have a rather high bactericidal effect. Viruses are very quickly inactivated under the influence of ultraviolet rays with a wave length of 260-300 mμ. These waves are

absorbed by the nucleic acid of viruses. Longer waves are weaker and do not render viruses harmless.

Viruses in comparison to bacteria are less resistant to X-rays, and gamma-rays. Beta-rays are more markedly viricidal. Alpha-, beta- and gamma-rays in small doses enhance multiplication but in large doses they are lethal to microbes. Viruses which are pathogenic to animals are inactivated by 44,000-280,000 roentgens. Thiobacteria which live in uranium ore deposits are highly resistant to radioactive rays.

As to the mechanism of the effect of ionizing radiation, there are two hypotheses. According to the first, ionizing radiation has a direct effect on the microorganisms as a result of direct absorption of the radiation energy. According to supporters of the second hypothesis, ionizing radiation has an indirect effect due to the interaction of proteins with activated molecules of water and the production of free radicals.

It has been established that the direct effect of ionizing radiation first of all damages the nucleic acids of bacteria and viruses which carry the genetic information, and causes profound changes in the microorganisms or kills them. However, considering the presence of a large amount of water (75-85 per cent) in the microbial cell of greatest importance in the mechanism of the effect of radioactive matter is the production of reactive radicals which when interacting with cellular proteins cause an intense oxidation process and break down living matter.

Ionizing radiation can be used for practical purposes in sterilizing food products, and this method of cold sterilization has a number of advantages. The quality of the product is not changed as during heat sterilization which causes denaturation of its component parts (proteins, polysaccharides, vitamins). Radiation sterilization can be applied in the practice of treating biological preparations (vaccines, sera, phages, etc.).

Of interest is the phenomenon of *photoreactivation*, described in 1949 by A. Kelner. If a suspension of bacteria is preliminarily exposed to visible light radiation it becomes more resistant to ultraviolet radiation. If after exposure to strong ultraviolet light a suspension of colibacilli is irradiated with visible light marked growth of the bacteria is observed when they are seeded on nutrient media. It has been proposed that under the influence of visible light irradiation factors are produced which render harmless the action of toxic substances produced under the effect of ultraviolet rays. The reactivation of bacteria is possible as a result of repeated heating at 46.5°C, and also by placing the irradiated culture in darkness. The ability to reactivate viruses as proposed is connected with the preservation of the native state of DNA.

The effect of high pressure and mechanical injury on microbes. Bacteria withstand easily atmospheric pressure. They do not notice-

ably alter at pressures from 100 to 900 atm at marine and oceanic depths of 1,000-10,000 m. Yeasts retain their viability at a pressure of 500 atm. Some bacteria, yeasts and moulds withstand a pressure of 3,000 atm and phytopathogenic viruses withstand 5,000 atm.

The movement of liquid media has a harmful effect on microbes. The movement of water in rivers and streams, undulations in stagnant waters are factors important in self-purification of reservoirs from microbes.

Ultrasonic oscillation (waves with a frequency of about 20,000 hertz per second) has bactericidal properties. At present this is used for the sterilization of food products, for the preparation of vaccines and the disinfection of various objects.

The mechanism of the bactericidal action of ultrasonic oscillation is that in the cytoplasm of bacteria found in an aquatic medium a cavity is formed which is filled with liquid vapours. A pressure of 10,000 atmospheres occurs in the bubble, which leads to disintegration of the cytoplasmic structures. It is possible that highly reactive hydroxyl radicals originate in the cavities formed in the sonified water medium.

Of certain significance in rendering the air harmless is *aeroionization*. The negatively charged ions have a more lethal effect on the microbes.

THE EFFECT OF CHEMICAL FACTORS

Depending on the physicochemical composition of the medium, concentration, the length of contact and temperature chemical substances have a different influence on microbes. In small doses they act as stimulants, in bactericidal concentrations they paralyse the dehydrogenase activity of bacteria.

According to their effect on bacteria, bactericidal chemical substances can be subdivided into surface-active substances, dyes, phenols and their derivatives, salts of heavy metals, oxidizing agents and the formaldehyde group.

Surface-active substances change the energy ratio. Bacterial cells lose their negative charge and acquire a positive charge which impairs the normal function of the cytoplasmic membrane.

Bactericidal substances with surface-active action include fatty acids and soaps which harm only the cell wall and do not penetrate into the cell.

Phenol, cresol and related derivatives first of all injure the cell wall and then the cell proteins. Some substances of this group inhibit the function of the coenzyme (diphosphopiridine nucleotide) which participates in the dehydrogenation of glucose and lactic acid.

Dyes are able to inhibit the growth of bacteria. The basis of this action is the marked affinity for the phosphoric acid groups of nu-

cleoproteins. Dyes with bactericidal properties include brilliant green, rivanol, tripaflavine, acriflavine, etc.

Salts of heavy metals (lead, copper, zinc, silver, mercury) cause coagulation of the cell proteins. When the salts of the heavy metal interact with the protein a metallic albuminate and a free acid are produced.

$$R - COOH + AgNO_3 \rightarrow R - COOAg + HNO_3$$

A whole series of metals (silver, gold, copper, zinc, tin, lead, etc.) have an *oligodynamic* action (bactericidal capacity). Thus, for example, silverware, silver-plated objects, silver-plated sand in contact with water render the metal bactericidal to many species of bacteria. The mechanism of the oligodynamic action is that the positively charged metallic ions are adsorbed on the negatively charged bacterial surface, and alter the permeability of the cytoplasmic membrane. It is possible that during this process the nutrition and reproduction of bacteria are disturbed. Viruses also are quite sensitive to the salts of heavy metals under the influence of which they become irreversibly inactivated.

Oxidizing agents act on the sulphohydryl groups of active proteins. More powerful oxidizing agents are harmful also to other groups (phenol, thioethyl, indole, amine).

The following reaction takes place.

$$2R - CO - NH - R_1 + 2Cl_2 \rightarrow 2R - CO - NCl - R_1 + 2HCl$$

Oxidizing agents include chlorine which impairs dehydrogenases, hydrolases, amylases and proteinases of bacteria and which is widely used in decontaminating water, and chloride of lime and chloramine used as disinfectants. In medicine iodine is used successfully as an antimicrobial substance in the form of iodine tincture which not only oxidizes the active groups of the proteins of bacterial cytoplasm, but brings about their denaturation. Potassium permanganate, hydrogen peroxide and other substances also have oxidizing properties.

Many species of viruses are resistant to the action of ether, chloroform, ethyl and methyl alcohol and volatile oils. Almost all viruses survive for long periods in the presence of whole or 50 per cent glycerin solution in Ringer's and Tyrode's solutions. Viruses are destroyed by sodium hydroxide, potassium hydroxide, chloramine, chloride of lime, chlorine and other oxidizing agents.

Formaldehyde is used as a 40 per cent solution known as formalin. Its antimicrobial action can be explained, as presumed, by its being united to the amino groups of proteins which causes their denaturation. Formaldehyde kills both the vegetative forms as well as the spores. It is applied for decontaminating diphtheria and tetanus toxins as a result of which they are transformed into *anatoxins* (see p. 260). Some viruses (phages, tobacco mosaic virus) inactivated by formalin can sometimes renew their infectivity.

In nature microorganisms constitute a component of the biocoenosis (a community of plants and animals living in a part of the habitat with more or less homogenous conditions of life).

Microbes are found in nature in associations among which there is a constant struggle for existence. Certain species which adapted themselves to a given medium have more marked antagonistic properties in comparison to other species which have fallen into a new habitat. Thus, for example, lactic acid bacteria are antagonistic in relation to the causative agents of dysentery, plague, etc. Blue-pus bacteria inhibit the growth of dysentery, enteric fever microbes, anthrax bacilli, cholera vibrio, causative agents of plague, glanders, and staphylococci, meningococci, etc. The normal inhabitants of the human body (e.g., colibacilli, enterococci, lactobacilli, microflora of the skin and nasopharynx, etc.) have especially potent antagonistic properties.

For many years a controversy raged on the possibility of intraspecies antagonism among microbes. At the present time many scientists have established the antagonistic relationships not only between virulent and nonvirulent strains of the same species. These properties are found in certain strains of colibacilli, pneumococci, enteric fever and dysentery bacteria, staphylococci, etc.

In certain conditions antagonistic properties appear in microbes due to a lack of nutrients, as a result of which some microbes are forced to feed at the expense of others. This phenomenon was named forced antagonism by I. Schiller.

Antagonistic relations have been established in viruses when one virus protects the organism from penetration by another virus. In virology this has been called viral interference.

Among various groups of microbes there are several types of relationships: symbiosis, metabiosis, satellism, synergism and antagonism.

Symbiosis represents an intimate mutually beneficial relation of organisms of different species. They develop together better than separately. Sometimes the adaptation of two organisms becomes so profound that they lose their ability to exist separately (symbiosis of the fungus and blue-green alga, nitrogen-fixing bacteria and cellulose-decomposing bacteria, symbiosis of nodule bacteria with legume plants, various fungi with the roots of plants, yeast-like fungi and lamblias).

Metabiosis is that type of relationship in which one organism continues the process caused by another organism, liberating it from the products of life activities, and thus creating conditions for its further development (nitrifying and ammonizing bacteria).

During **satellism** one of the symbionts known as the favourable microbe incites the growth of the other (some yeasts and sarcinae

producing amino acids, vitamins, etc., enhance the growth of microbes more strict in relation to nutrient media).

Synergism is characterized by the increase in the physiological functions of the microbial association (yeasts, lactobacilli, fusobacteria and *Borrelia* organisms).

One of the forms of symbiosis is *a virus-carrying form*—a communal existence of some bacteria and protozoa with viruses (lysogenic bacteria retain the corresponding phages for long periods in their cells, during chronic tonsillitis besides the α-haemolytic streptococci, the adenoviruses take part in the infectious process, etc.).

During antagonistic relationships there is a struggle for oxygen, nutrients and a habitat. On the mechanism of antagonistic relationships see "Antibiotics".

Modern understanding of the problems of microbiology unfolds complex relationships among organisms and the essence of biological laws.

Biological factors have received widespread application in treating many infectious diseases with the products of the life activities of bacteria, fungi, higher plants, and animal tissues known as *antibiotics*. These effective drug preparations include penicillin, streptomycin, chlortetracycline, chloromycetin, tetracycline and many others.

In decontaminating the environment from pathogenic microorganisms by antagonism an important role is played by phages widespread in the soil and water and by *phytoncides*, volatile substances of many plants.

The influence of the environment is taken into account by the physician in combating harmful microorganisms (sterilization, disinfection), vectors of causative agents of infectious disease (disinsection) and rodents—reservoirs of pathogenic microorganisms (deratization).

Sterilization of instruments, needles, syringes, etc., is carried out by boiling. Dressings, glassware and salt solutions are treated in autoclaves.

Disinfection is carried out in several ways:

(1) *mechanical disinfection* (washing clothes, washing the hands, using a vacuum cleaner, ventilators, filtration, burying the cadavers of animals and humans, etc.);

(2) *physical disinfection* (boiling, dry heat, steam under pressure, dry heat and steam chambers);

(3) *chemical disinfection* (decontamination by using certain concentrations of soda, alkaline solution, soap, lime, chalk, limewater, chloride of lime, chloramine, phenol, cresol, formaldehyde, sulphur dioxide, chlorpicrine and other substances).

Antiseptics is of great significance in medical practice. The people of Africa in ancient times knew the methods of treating wounds with the aid of ant bites which healed the edges of the wound no worse than if it had been stitched by modern medical techniques. Sunlight took the place of antiseptic substances. Yet in 1865, N. Piro-

gov pointed out the necessity of destroying the source of intrahospital infection and tried chlorine water, silver nitrate, iodine and other antiseptic substances in combating wound suppurations. In 1867 J. Lister used phenol extensively as an antiseptic.

The science of antiseptics played a large role in the development of surgery. The practical application of microbiology in surgery brought a decrease in the number of postoperative complications, including gangrene, and to a considerable degree diminished the death rate in surgical wards.

J. Lister highly assessed the importance of antiseptics and the merits of L. Pasteur in this field.

This trend received further development after E. Bergman in 1897 introduced aseptics into surgical practice representing a whole system of measures directed at preventing the access of microbes into wounds. Aseptics is attained by disinfection of the air and equipment of the operating room, by sterilization of surgical instruments and material, and by disinfecting the hands of the surgeon and the skin on the operation field.

A widespread use of aseptics has permitted the maintainance of the health and lives of many millions of people.

Modern methods of aseptics have been perfected to a considerable extent, due to which almost all operations are accompanied by primary healing of wounds without suppuration, while the incidence of postoperative septicaemia has been completely eliminated.

BACTERIOPHAGES

Bacteriophages are bacterial viruses which pass through minute pore filters and live at the expense of reproducing bacteria. Bacteriophagia is the process of interaction of the bacteriophage with bacteria.

In 1898 N. Gamaleia examined the solution of anthrax bacilli, cholera vibrios and streptococci in distilled water and named the factor providing for the lysis of bacteria bacteriolysin.

In 1915 the English bacteriologist F. Twort noticed a change in *Staphylococcus albus* colonies which became glassy and transparent. Reinoculation of these colonies produced no growth or only single colonies developed which gradually became glassy. If a very small amount of these colonies was transferred to another normal colony the latter was changed. When the infected culture was seeded many glassy colonies appeared together with the normal colonies.

F. Twort suggested that the production of glassy colonies in a staphylococcal culture appears to be an acute infectious disease in microbes. Upon investigating the specimens of glassy colonies under the microscope no normal cocci were observed.

On the basis of data received, F. Twort considered the active source causing the disease in staphylococci to be a virus which is widespread in nature. F. Twort assumed also that this established factor was an autolytic enzyme.

In 1917 F. d'Herelle published analogous observations on the causative agent of dysentery. For the sake of determining the reasons for the decrease in number of dysentery bacteria excreted in the faeces of convalescents, he seeded daily the faeces of dysentery patients into test tubes of broth. On the following day he filtered the contents of the test tubes through china candle filters, and a few drops of filtrate were added to the young broth culture of dysentery bacteria. During the first days of illness the seeded dysentery bacteria in the culture developed constantly, but at the termination of the disease the broth with the culture to which the filtrate of the faeces was added remained transparent. This coincided with the time of convalescence. The filtrate of the faeces of a convalescent added to a broth culture of the causative agent of dysentery caused an inhibition of its multiplication. After 3-4 successive passages the lytic activity of the filtrate increased enormously. In a 10^{-12} dilution it was capable of inhibiting the growth of a freshly seeded broth culture.

Thus, the agent studied by F. d'Herelle was capable of destroying dysentery bacteria and was found in the faeces of dysentery convalescents. It was also able to multiply upon interaction with growing dysentery microbes. It was named a bacteriophage. At present when this phenomenon has been discovered not only in bacteria but also in actinomycetes and blue-green algae, scientists considered it advisable to substitute the term "bacteriophage" by "phage".

The phenomenon of bacteriophagia is easily observed not only in liquid, but also on solid nutrient media. If a few drops of the corresponding highly concentrated phage are introduced into a dish with a nutrient medium seeded with a culture, then on the next day no growth will occur at the point of introduction of the phage (Fig. 43).

If a less concentrated phage is added to the culture then areas free from bacterial growth (phage colonies) are produced as a result of the destruction of bacteria only at those points where the phage corpuscles had access (Fig. 44). These zones of lysis were called by

Fig. 43. Lysogenic action of the phage on a solid medium

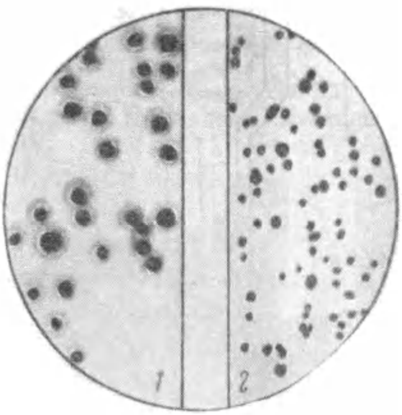


Fig. 44. Phage colonies in a dish with a nutrient medium seeded with *E. coli*

1—large colonies;
2—small colonies

French scientists as taches vièrges (virgin spots), in Germany they received the name of Löcher (bald patches).

The investigations of F. d'Herelle on phage laid the foundations for a more detailed study of a new section in microbiology.

Main properties of phages. *Morphology.* In appearance phages are tadpole- or spermatozoid-shaped (Fig. 45). The head of the phage has a wall and is filled with granular contents. The sizes of the heads of bacterial phages studied vary from 47 to 104 mμ and the tails are from 10 to 225 mμ. The phages of colibacilli have a head 65-95 mμ and a tail 25-100 mμ. Phages for blue-green algae do not have tails.

Upon studying ultrathin sections of phage particles it was established that the head consists of a double wall: the outer and the thin, inner phagoplasmatic membrane which encloses the contents of the head.

Chemical composition. The complex structures of the organic compounds of phages and their corresponding bacteria are different. The contents of the head of the phage are made up of DNA which has a spiral structure and is highly polymerized. The phage DNA differs chemically from the bacterial DNA. By means of osmotic shock (a sudden decrease in salt concentration) the DNA is freed from the phage particles, and phage shadows remain. The molecular weight of the phage DNA of the colibacillus is equal to 25,000,000.

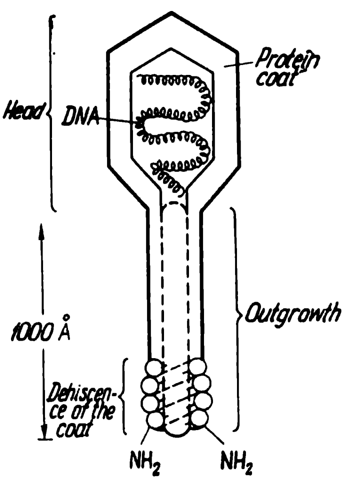


Fig. 45. Diagram of the structure of a phage

In addition, the phage of colibacillus contains protein found in the wall of the phage particle and a small amount of lipids. The protein content is 50-60 per cent, that of desoxyribonucleic acid is 40-50 per cent, and that of lipids (in the form of neutral fats)—1.7-2.6 per cent. In other bacterial phages these ratios are different.

The resistance of phages to physical and chemical factors is greater than that of the corresponding microbes. Phages withstand high pressures (up to 6,000 atmospheres), are resistant to the action of radiant energy, and maintain their activity in a pH range from 2.5 to 8.5. In sealed ampoules phages do not lose their lytic properties for 5-6 and even 12-13 years and can be preserved for relatively long periods in glycerin. Phages perish quickly under the action of boiling, acids, ultraviolet rays and chemical disinfectants. In relation to resistance phages are intermediate between the vegetative forms of bacteria and spores.

Some substances (thymol, chloroform) and enzyme poisons (cyanide, fluoride, dinitrophenol) have no effect on phages, but cause bacteria to perish or inhibit their growth. These preparations are used for maintaining phages in cultures and for destroying bacteria, actinomycetes and fungi.

The specific action of phages. Numerous investigations have established that phages have a strict species as well as type specificity. The typhoid fever phage develops at the expense of typhoid fever bacteria, the dysentery phage at the expense of the dysentery bacteria, etc. In the majority of cases the given phage has no effect on other species or types of bacteria. Thus, for example, types T1, T3 and T7 colibacillus phages produce large colonies on solid nutrient media, types T2, T4 and T6 produce small colonies. Morphologically

these types are also different. Phages have a great adaptive potency. One species of phage can be "taught" to act upon other species of bacteria by gradual and successive passages.

Classification of phages. Naturally the necessity of introducing taxonomy has arisen from the standard conception on the nature of phages as living matter. However, up to the present time this problem has not been solved. Many different principles of classification of the phages have been proposed taking into consideration size, morphology, chemical composition, the ability to take part in the neutralization reaction with antiphage serum, specific action on various species and types of bacteria, and other characters. However, these characters are not stable and they are not true criteria for working out a phage classification according to Linneaus' principle of binomial nomenclature.

Mechanism of the interaction of phage and bacteria. The bacteriophage phenomenon depends on the age of the culture, the concentration of bacteria, phage activity, bacterial phage resistance, composition of the nutrient medium, temperature and other factors.

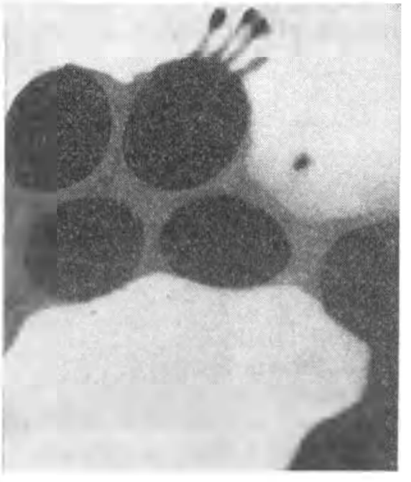


Fig. 46. The interaction of the phage with *Staphylococcus aureus* cells

When the phage particles come into contact with the bacterial cell they are adsorbed on its surface (Fig. 46).

Phage adsorption on the surface of the cell takes place as a result of the interaction of the amino groups of proteins localized at the margin of the phage tail, and the negatively charged carboxyl groups on the surface of bacterial cells.

There are reversible and irreversible phases of adsorption. *The reversible phase* is characterized by the fact that the attached phage particles can be separated from the cell by a vigorous agitation or a sudden decrease in ion concentration. The liberated phage particles retain their viability.

The adsorption phase depends on a number of factors (composition and viscosity of medium, temperature). In distilled water phage adsorption does not take place. The presence of bivalent cations (Ca^{++} and Mg^{++}) stimulates the process of adsorption. Adsorption stimulators include triptophane and isoleucine. Citrates, indole, etc., inhibit the process of phage adsorption.

After the first phase follows the second, *irreversible* adsorption phase after which the phage does not separate from the bacterial cell. Due to the presence of an enzyme of the lysozyme type the phage penetrates the bacterial cell through a perforation formed in the cell wall.

According to many investigations, the phage is adsorbed on the bacteria by its tail end which penetrates the bacterial cell wall. Then the granules of the tail and contents of the head in the shape of a tightly coiled spiral of DNA flow into the bacterial cell, while the cell wall remains outside as an empty "coat". Once in the bacterial cell the phage DNA inhibits the genetic function of bacterial DNA and provides for the synthesis of phage particles. This process takes place according to a certain plan. At the end of the latent period the production of phages of the bacterial cell commences, and the cell wall is disrupted (Fig. 47). The whole cycle of phage development is completed within 30-90 minutes. This above mentioned mechanism of the interaction of the phage with the microbial cell is known as *inner lysis of bacteria* when the cell wall ruptures, with the release of the phage and the lysis of the bacteria. *External lysis*

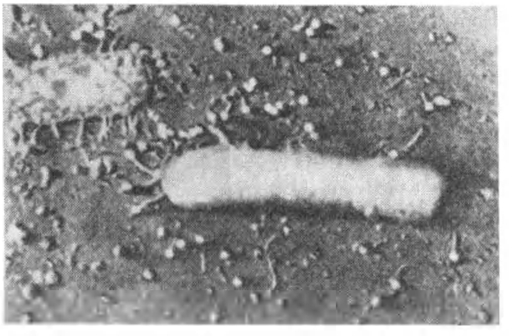


Fig. 47. A bacterial cell destroyed by a phage. Multiplying phages

of bacteria is not accompanied by the production of new phages, but under the influence of lytic enzymes of a large number of adsorbed phages (more than 100) at different sites of the cell the bacterial wall dissolves and lysis of the cell occurs.

According to the nature of interaction with the bacterial cell there are virulent phages, temperate phages and an intermediate group of phages.

Virulent phages bring about the production of new phages and the lysis of the bacterial cell.

The phage nucleic acid which has penetrated into the bacterial cell brings about the transformation of protein structures into the corresponding phage particles. This process is carried out under the influence of the enzymatic system of the bacteria. It has been suggested that the synthesis of nucleic acid and phage protein is carried out from the low molecular cellular compounds and not from ready-made complex substances.

In infected phage bacteria (Fig. 48) 7-8 phage particles are formed per minute.

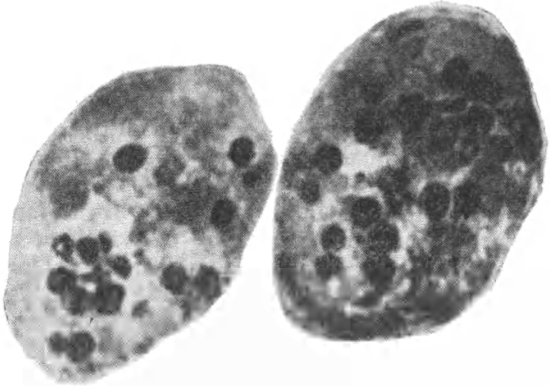


Fig. 48. Superthin sections of *E. coli* cells infected with a phage

The penetration of the phage into the cell is not always accompanied by its disintegration. In a number of cases the interaction of phage and bacteria leads to the formation of the so-called lysogenic cultures in which the phages as well as the bacteria are retained. This existence of two live systems can continue for a long time. The bacteria become carriers of phage particles and acquire resistance (phage resistance) to virulent phages. *Lysogeny* is caused by temperate phages. Temperate phage DNA resembles the bacterial DNA in structure due to which mutual adaptation of both takes place.

The intermediate group of phages includes the mutants of temperate phages (by means of which lysogeny is lost) and the mutants of virulent phages of a low virulence.

The phage is a potent factor of variation in bacteria and actinomycetes. Under its influence *transduction* (see chapter "Genetics of Microorganisms") and *lysogenization* occur due to which bacteria acquire the property of lysogeny. Upon infecting sensitive bacterial strains with temperate phages a fraction of the cells is lysed with the formation of a vegetative phage. The other part of the cells survives and becomes lysogenic. In lysogenic bacteria the temperate phage transforms into the prophage which is incapable of causing

bacterial lysis. Lysogenic bacteria occurring as a result of lysogenization become phage carriers for a long time and acquire immunity to this type of phage. The ratio of the amount of lysogenic cells produced when the culture is infected with a temperate phage to the total number of infected cells is known as the frequency of lysogenization. The frequency of lysogenization depends on the conditions of infection and the genetic structure of the phage. Low temperatures are favourable to lysogenization, while high temperatures are inhibitory.

Parallel with the formation of new microbe variants with a high adaptive potency, the phage plays a large part in the evolution of microbes. New forms, in turn, are the source of the formation of variants, and this process is continuous in nature.

At present the phage is widely used as a model for studying problems in genetics. With its help a more accurate differentiation of the processes of variation and heredity can be made.

The phage in the human body in some cases acts on the microbes in such a way that the pathogenic properties of microorganisms are altered, their virulence and toxicity are diminished, and their action as harmful irritants is decreased, and thus enables the defense forces to free completely the body from the causative agents.

The nature of the phage. N. Gamaleia pointed out that phage multiplication and adaptation, corpuscularity and other properties inherent to living matter make it possible to regard phages as viruses. D'Herelle considered the phage to be noncellular matter. The virus theory of the origin of the phage is the most well founded. Phages are widely used in medical practice (prophylaxis, therapy and diagnosis of infectious diseases). Antiphage sera are used for combating phages of industrial cultures, employed in the preparation of vaccines and antibiotics.

At present the problem of the exogenic or endogenic origin of phages holds interest. Due to the study of lysogeny, it was revealed that in lysogenic bacteria the phage occurs in a certain form which has been named prophage. This phage structure penetrates into the cell nucleus and makes it lysogenic. The prophage does not possess infectious properties. It has been suggested that the state of lysogeny occurs as a result of joining the hereditary determinants of the phage and bacteria. This bond is stable, and is impaired by the effect of ultraviolet light, ionizing radiation and chemical preparations on the lysogenic bacteria. Under the influence of the above mentioned substances, named inducers, the prophage becomes a vegetative phage, while the bacteria lose their lysogenic state.

Distribution of phages in nature. Phages are wide-spread in nature. Wherever bacteria are found—in the animal body, in body secretions, in water, drainage waters and in museum cultures, conditions may be created for the development of phages. Specific phages have been found in the intestine of animals, birds, humans, and also in

galls of plants, and in nodules and legumes. Phage has been isolated from milk, vegetables and fruits.

River water, sea water and drainage waters quite frequently contain an abundance of phages in relation to various microbes including pathogenic (cholera vibrio, bacteria of enteric fever, paratyphoid. dysentery) organisms.

Sick people and animals, carriers and convalescents serve as the main source of phages against pathogenic microbes. In sick people the phage can be found not only in the intestinal contents, but in the urine, blood, sputum, saliva, pus, nasal exudate, etc. It is extremely easy to isolate the phage during the period of convalescence. The phage is employed for the determination of species and type specificity of the isolated cultures. This method has been named phage diagnostics.

The discovery of different phages against pathogenic microbes in the environment (water, soil) illustrates the presence in a given area of sick people and animals which excrete the corresponding agents or phages. This can be employed in giving an additional characteristic of the sanitary-epidemic state of water sources and the soil.

The isolation of the phage from the material under investigation has been carried out by a special direct method and an accumulation method. F. Sergienko, G. Katsnelson and M. Sutton, V. Timakov and D. Goldfarb devised a method for the rapid discovery of pathogenic bacteria in the environment with the help of the reaction of successive growth of the titre of the specific phage (RGP).

Production of a phage and the determination of its activity. The phage is obtained by adding a special maternal phage into vats with broth cultures, which are kept for one day at 37°C and then filtered. The filtrate is checked for purity, sterility, harmlessness and activity (potency).

Practical importance of the phage in medicine. Arising from the data obtained on the mechanism of phage activity, phages have been used in prophylaxis and medical treatment against dysentery, enteric fever, paratyphoid, cholera, plague, anaerobic, staphylococcal, streptococcal and other diseases.

Phage prophylaxis and phage therapy are carried out in combination with other methods.

The dysenteric polyvalent and choleric phages are used for prophylaxis and treatment. Enteric fever phage is prepared against O- and Vi-strains of enteric fever bacteria and is used according to epidemic indices for prophylaxis. Anaerobic phages are introduced for the therapy of gas gangrene. The diphage (staphylococcus and streptococcus) is employed for prevention and treatment of suppurative wounds.

In the practice of phage application the degree of phage sensitivity of the causative agents of infectious diseases is taken into account.

Bacteriophagia is used in the diagnosis of certain infectious diseases. With the help of special phages the species and types of isolated bacteria of the typhoid-dysentery group, staphylococci, causative agents of plague, cholera, etc., are determined.

Phages are often very harmful in the manufacture of antibiotics and sour milk products as a result of inhibiting beneficial microorganisms.

At present due to the introduction of antibiotics into practice phage therapy of infectious disease is used to a limited extent, while phage prophylaxis is carried out according to epidemic indices only in the case of a small number of infectious diseases (dysentery, enteric fever, cholera).

Due to the development of space microbiology lysogenic cultures are used as radiation detectors under the influence of which the temperate phage is transformed into the vegetative form with successive bacterial lysis, and the release of mature phages. Thus, for example. in the second Soviet spaceship "Vostok-2" there were lysogenic strains of the colibacilli which are highly sensitive to ionizing radiation. Under the effect of 1 roentgen they are capable of producing a vegetative phage, while nonlysogenic bacteria can withstand tens of thousands of roentgens without any profound changes. For studying outer space other viruses are employed in addition to phages.

GENETICS OF MICROORGANISMS

Three periods can be observed in the history of the development of genetics (the study of heredity and variation) of microorganisms. In the first, which includes the second half of the 19th century, certain species of microbes were isolated and described. The second period (1900-1940) was characterized by the discovery and study of a large number of bacterial varieties differing in a whole series of characters. The third period began in 1940-1944, the characteristic feature of which was the systematization of the data received, and the rapid development in the field of the genetics of bacteria and viruses which was of quite great significance both theoretically and practically.

The history of the development of the study of the variation of organisms is the history of the battle of two outlooks in biology — materialistic and idealistic. The essence of this battle is that one trend upholds the idea of the fixity of the species created by a creator (Aristotle, Linnaeus, etc.), while the supporters of another view unfolded the evolutionary science in the development of living beings (Democritus, J. Lamarck, Ch. Darwin, etc.).

In microbiology also some scientists (F. Cohn, R. Koch et al) adhered to the concept of *monomorphism*, according to which microbes have a constancy of properties and species. Others developed the conception receiving the name of *pleomorphism*, which confirmed a wide range of variations and species formation (G. Buchner, V. Tsopf. C. Nägeli, E. Metchnikoff, L. Pasteur, L. Tsenkovsky, M. Beijerinck, etc.).

In the latter half of the XIX century there was a stubborn struggle between the advocates of these conceptions, which terminated in the victory of the theory of monomorphism.

At that time the science of monomorphism, in spite of its erroneous theoretical basis, played a positive role. It allowed the investigation of the specific properties of microbes, the development of laboratory diagnostic techniques, the elucidation and study of the

causative agents of many infectious diseases. However, during the development of microbiology facts began to accumulate which gave evidence for the different changes taking place in microbes.

Later monomorphism became a stagnant dogma of a metaphysical trend in microbiology, and gradually began to lose its position to the modern ideas reflecting the evolutionary theory, which correctly explained the reasons for the transformation of living forms.

According to Ch. Darwin, the evolution of any group of organisms takes place through the evolution of adaptation to environmental conditions. Thus, the most important factor of the evolutionary process is the adaptive nature of the variation of organisms. That is why all kinds of antidarwinian conceptions could not influence Darwin's theory of evolution, including the mechanistic Lamarckian theory in which the evolutionary process was regarded as a direct result of the reaction of the organism to the physicochemical effects of the environment. According to the assertion of the adherents of the mechanistic Lamarckian theory, a long-term effect of environmental factors brings about a change in the organisms, while these changes become hereditary. That is why they confirmed the ability of inheriting favourable acquired characters. At the same time no explanation is given as to why the organism reacts adequately to the effects of the environment. In fact, the organism is adapted to the environment, because it changes adaptively. The adherents of the mechanistic Lamarckian theory accept the inner force of expediency as the main property of live organisms, and thus support idealism. Its modern contemporaries have brought nothing new into the science of the evolution of organisms.

The theory of neodarwinism of A. Weismann arose in the early stages of development of genetics. Weismann did not deny Darwin's theory, but exaggerated the role of natural selection and developed a theory based on the "omnipotence" of selection. He considered the organism to be a mosaic of parts, and the genotype, a hereditary basis of the organism, as a mosaic of independent determinants. According to A. Weismann, there is a struggle for existence among the determinants, the consequence of which is "embryonic selection" which determines the processes of variation and trend of the species.

J. Lotze's conception develops further the Weismann theory. He attempted to demonstrate that evolution can be boiled down to the recombination of the given gene complex. New species were considered to be a result of the crossing-over of the forms which existed or those which are existing. Changes in the genotypes themselves do not take place. This point of view was named the theory of evolution with constancy of species.

The mutation theory of de Fries (1901) explains the origin of new forms by mutations, which occur autonomously and independently of the environment in the premutation period, when inner

factors or creative forces accumulate in the body. New species formed as a result of mutations are exposed later to the eliminating effect of selection. The idealistic nature of this conception is that the adaptation of species to the conditions of life is not the consequence of natural selection, but the result of the mutation process which creates harmonic forms independently.

The theory of evolution was created in conditions of the continual struggle between materialism and idealism during a long period of time, and with each stage it was supplemented with new data.

VARIATION OF THE MAIN CHARACTERS OF MICROORGANISMS

Changes in morphological characters. Under the influence of physical and chemical effects some individuals assume the form of large spheres, thickened filaments, flask-shaped formations, and branchings resembling fungal mycelia. Acetic acid bacteria under the influence of a temperature of 41°C easily form very long, strongly swollen filaments. The cultivation of these bacteria at ordinary temperatures is accompanied by the appearance of rod-shaped forms.

N. Gamaleia observed morphological changes in a number of microbes, e.g., the formation of giant spheres, amoeboid forms, thickened filaments, etc. He named this phenomenon *heteromorphism* which arises due to the adaptation of bacteria to unusual environmental conditions.

Heteromorphism easily occurs under the influence of lithium salts, phage, caffeine, sulphonamides, antibiotics, different types of irradiation and also many other factors.

The phenomenon of heteromorphism is relatively often observed when the culture ages. Fig. 49 illustrates the elongated forms of cocci (1), filamentous forms of the colibacilli (2), filamentous forms and branched forms of tubercle bacilli (3-4); figures 50-51 show the flask-shaped, filamentous, yeast-like and coccus-like forms of diphtheria bacilli. The variation of morphological forms is most distinctly expressed in mycoplasmas and L-forms of bacteria (see p. 44).

A number of other characters (affinity to dyes, formation of flagella, cilia, spores, capsules, structures of the nuclear apparatus) are exposed to variation.

It should be noted that any change in morphological characters is accompanied by a change in physiological properties. That is why the subdivision of the types of bacterial variation into morphological, cultural, fermentative, biological, etc., is arbitrary, and is done for the purpose of illustrating better the variety of material in this problem.

Changes in cultural properties. Besides morphological deviations in microbes, changes are often observed in the cultural properties.

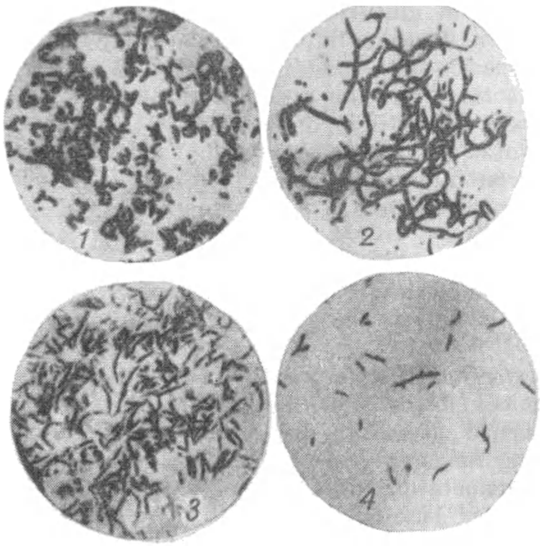


Fig. 49. Variation in morphology of bacteria

1—formation of elongated forms of cocci; *2*—filamentous forms of *E. coli*; *3*—filamentous forms of tubercle bacilli; *4*—branchings in tubercle bacilli

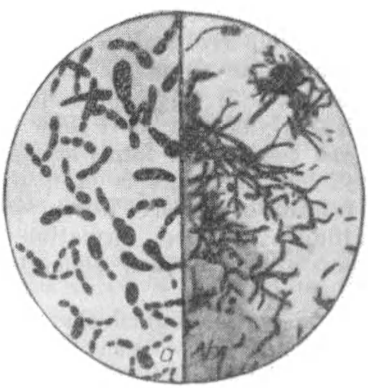


Fig. 50. Diphtheria bacilli

a—flask-shaped form; *b*—filamentous form

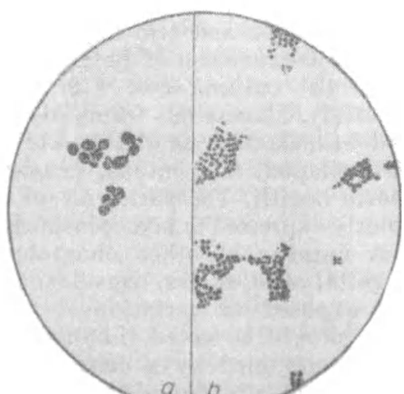


Fig. 51. Diphtheria bacilli

a—yeast-like form; *b*—coccus-like form

In 1920-1924 P. De Kruif, G. Arkwright and many other scientists established that the cultures of one and the same species of bacteria may differ among themselves. When a pure culture is seeded onto a solid nutrient medium different forms of colonies of two main types are produced: (1) smooth, S-forms, and (2) rough, R-forms.

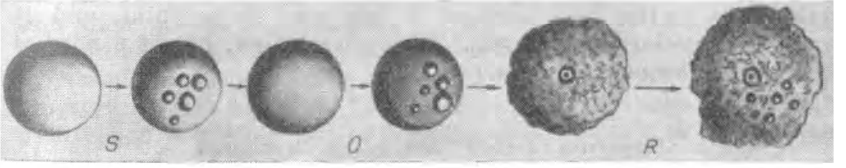


Fig. 52. Altered forms of colonies in the process of bacterial dissociation
S—smooth; O—opaque; R—rough

Between these two types of colonies there are transitional, unstable forms and more often O-forms (Fig. 52). The difference between S- and R-forms is not only limited to the forms of the colonies, but in-

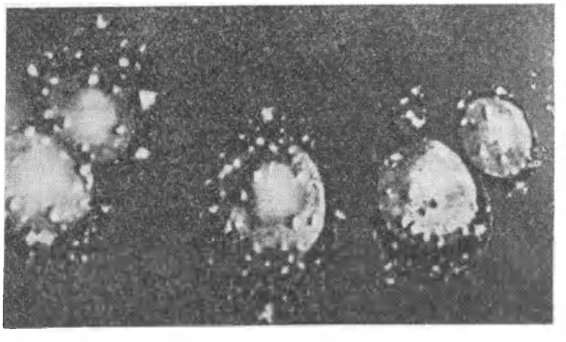


Fig. 53. Formation of daughter colonies in the process of variation in *Staphylococcus aureus*

cludes other characters. This kind of variation is known as *dissociation* (Table 8). In some cases the formation of daughter colonies is observed (Fig. 53). There are other forms of colonies: D-colonies (dwarf) and G-colonies (gonidial) which originate on the surface or margin of normal colonies and L-colonies (see p. 44).

Some microbiologists attributed all types of variation to dissociation which was considered to be the sole form of variation

in microbes, and regarded this process as movement within a closed cycle of bacterial development.

$$
\begin{tikzcd}
 & S \arrow[dr] & \\
R \arrow[ur] & & O \arrow[dl] \\
 & M \arrow[ul] &
\end{tikzcd}
$$

According to F. Hedley there are three forms which with a certain conformity replace one another: M (mucous), S (smooth), and R (rough). Dissociation is not regarded as a variation, but as a normal cycle of bacterial development.

Table 8

Properties of Cells from S- and R-Colonies

S-type	R-type
Uniform turbidity when grown in broth	Growth in broth forms a precipitate
Suspension in 0.85 per cent saline solution is homogenous and stable	Suspension in 0.85 per cent saline solution is precipitated
Uniform luxurious growth on agar slant	Growth on agar is dense, dry, and rough
Colonies smooth, regular, convex	Colonies rough, uneven, flattened
Colonies form daughter buds. Motile species possess flagella	Rarely form daughter buds. Flagella may be absent in motile species
Well developed capsules in capsulated species	Capsules are absent
Biochemically more active	Biochemically less active
Complete antigenically	Often has unspecific antigen
Contains a specific polysaccharide	Contains unspecific polysaccharide
Flake loose precipitate in immune serum	Minute granular precipitate in immune serum
Majority of pathogenic species has a virulent stage	Weak or no virulent stage
Usually separates in the acute period of disease	Associated mostly with the chronic forms of diseases and carriers
Sensitive to phage	Less sensitive to phage
Cells of normal morphology	Apt to form short and coccus-shaped forms
In S-immune serum can change into O- and R-forms	No change in S-immune serum
No dissociation in R-immune serum	Dissociation in R-immune serum
Poorly phagocytable	Easily phagocytable

Variation in requirement in metabolites. Under the influence of antibiotics and chemotherapeutic substances, X-rays, ultraviolet irradiation and other effects, in some microbes the need of certain amino acids and growth factors appears which the original cultures did not require. These varieties which for their development require special conditions are known as *auxotrophic* in contrast to original strains—prototrophic. Thus auxotrophs differ from prototrophs in

that part of their metabolic processes is blocked, and they lack the ability of synthesizing the necessary metabolites. Thus, for example, after the effect of X-rays on *E. coli*, it began to require for its growth many factors (casein hydrolysate or yeast extract), while the original strain could develop in a synthetic medium in which amino acids and vitamins (minimal medium) were absent. Auxotrophic variants lost the ability to synthesize leucine or vitamin B_1, and their growth depended on the presence of a certain substrate containing the amino acid leucine and vitamin B_1.

One of the means of revealing auxotrophic variants is the use of penicillin which has a bactericidal effect only on cells in the state of division. Blocking can be reproduced in relation to tryptophan, atranil acid and other factors.

Physical and chemical factors can induce different changes in the ability to synthesize important metabolites in bacterial cultures. These changes take place under the influence of the mechanisms concerned with genetic information carried by DNA or RNA.

Variation in enzymatic functions. Variation in microbes is not limited to the morphology, size or cultural characters, but includes other properties. Of special theoretical and practical interest is the variation of enzymatic ability in bacteria and their adaptation to the changed internal and external environmental conditions.

G. Vortman (1882) and E. Duclaux (1883, 1899) established the changes in the production of enzymes in microbes under the influence of their habitat.

In 1906 N. Neisser and in 1907 R. Massini revealed a variant in *E. coli* which did not ferment lactose, and which they named *E. coli mutabile.* The following interesting phenomenon was observed. When a culture of this variant was seeded on Endo's medium containing lactose, bacteria developed as colourless colonies unable to ferment the milk sugar. However, within a few days small red daughter colonies which were composed of *E. coli* cells capable of fermenting lactose appeared on the surface of these colonies.

If a culture of daughter colonies is seeded on Endo's medium, then only *E. coli* colonies appear, coloured red due to the fermentation of lactose. Meanwhile, parent colonies plated onto Endo's medium give colourless colonies of *E. coli* again which do not ferment lactose, and daughter colonies of bacteria which ferment lactose. This conformity can be repeated in innumerous seedings with the same result.

In 1910 R. Burri established that the colibacillus can acquire the ability to ferment saccharose. By employing the phenomenon of training, strains of enteric fever bacilli adapted to dulcitol, saccharose, rhamnose and raffinose were obtained.

Similar adapted forms to sources of hydrocarbon, as well as to nitrogen and salt nutrition, were obtained with lactic acid streptococci, bacteria and in bacilli. N. Krasilnikov obtained different

forms of adaptation in actinomycetes. It is more difficult to obtain adapted forms with amylolytic and proteolytic enzymes.

The catalytic activity of bacteria can be increased many times by adding substrates inducing the synthesis of enzymes in the corresponding conditions of cultivation (certain amount of vitamins, pH level and degree of aeration).

In the course of time more data were accumulated confirming the possibility of artificially obtaining variants which have lost or acquired the ability to ferment particular substances. Thus, for example, yeasts were obtained which upon cultivation in the presence of cyanide lost their ability to produce cytochromoxidase, but acquired another oxidative mechanism, and their flavine content increased.

If this carbohydrate is added into a medium containing a culture of colibacillus which has lost its ability to absorb galactose, then growth is arrested due to the formation of galactose-1-phosphate in the bacterial cells.

By the action of certain toxic substances on bacteria it is possible to deprive them of their ability to produce various enzymes. The cultivation of *Clostridium perfringens* on a medium with a low content of iron brings about a decrease in the enzymatic ability of the microbe. Thus, the enzymatic activity is variable, and can be lost or gained. The loss of enzymatic activity may be constant or temporary.

The problem arises, in what way the microbial cell acquires new fermentative properties and adaptates itself to the new conditions of the medium. Some scientists explain the appearance of new enzymes as a result of preadaptation, while others consider that mutations and recombinations lie at the basis of this process. The formation of adaptive enzymes occurs not only at the expense of proenzymes found in the cell, which have not revealed their action, but as the result of the formation of new enzymes which were previously absent in the cell.

A decisive role belongs to the free amino acids. However, they can be produced at the expense of some ready-made proteins found in the cell and named precursors of adaptive enzymes.

Under the influence of an unusual metabolite (inducer or repressor) induction or repression of the synthesis of a definite enzyme takes place. The production of enzymes (specific proteins) takes place according to genetic processes in the cell. They are governed by the general laws of protein synthesis as a result of the interaction of DNA and RNA.

Of great interest is the problem of the possibility of the production of adaptive enzymes not only in intact cells, but in their protoplasts.

The production and disappearance of adaptive enzymes, as numerous investigations have shown, is a widespread phenomenon among microorganisms.

The variation of biological properties. A rather important circumstance is that in pathogenic microbes under the influence of different factors, the degree of pathogenicity is altered. The decrease in pathogenicity of microbes while retaining the ability to cause immunity was noticed long ago. L. Pasteur was the first to succeed in reproducing these properties in 1881. According to this principle, but with different modifications, altered forms of pathogenic microbes were obtained, named at first attenuated (weakened), and then live vaccines. This first stage of directed variation of microbes had a great influence on the further development of microbiology, and the study of the variation of microbes.

While working with microbes of chicken cholera, L.Pasteur left the cultures without seeding them for a long period of time. When he began his next experiments, on testing the cultures, he was surprised by their unusual properties. The microbes of chicken cholera had lost their pathogenicity, but were able to provoke immunity in chickens to infection with pathogenic cultures. From these observations L. Pasteur came to the conclusion that under the influence of different environmental factors, bacteria are able to lose their pathogenic properties, and at the same time retain the ability to produce specific immunity in the animal organism.

In 1881 L. Pasteur devised a method of preparing live vaccine against anthrax. He obtained it by exposing a culture of anthrax bacilli to a high temperature. During 12-24 days cultivation at 42-43°C anthrax bacilli lost the ability to cause the disease in animals when injected subcutaneously, but retained their immunogenic properties.

In Russia L. Tsenkovsky employing Pasteur's general principle produced a live antianthrax vaccine consisting of a suspension of weakened spores of anthrax bacilli.

In 1885 L. Pasteur by a sequence of 133 subinoculations in rabbit brain weakened the causative agent of rabies. L. Pasteur named the altered virus a fixed one (virus fixé). It proved to be harmless when injected subcutaneously, and at the same time was capable of preventing the development of rabies in animals and humans bitten by rabid animals.

A. Calmette and C. Guérin in France in 1919 reproduced a variety of the bovine species of tubercle bacilli by many passages on a potato medium with bile and glycerin at a temperature of 38°C. Seedings were made every 14 days; during 13 years 230 seedings were made. The weakened strain of tubercle bacilli of the bovine variety was named the BCG vaccine (BCG—French—Bacille Calmette-Guérin). It is employed successfully in vaccinating humans against tuberculosis.

Arising from the general principles of the variation of microbes, in 1925 B. Cotton, J. Beck and H. Smith in the USA, as a result of a six year cultivation of a bovine species of brucella on a potato

medium, obtained an altered strain which they named No. 19. This culture has lost its pathogenic properties to a certain degree, and is employed as a vaccine for inoculating animals and humans against brucellosis.

G. Girard and J. Robic in 1931 attained profound and stable changes in plague bacteria as a result of a five year cultivation on meat-peptone agar at a temperature of 16-20°C. This strain EV was used for the preparation of live vaccine against plague (the name was given after the initials of a girl who died from plague and from whom the culture was isolated).

In 1936 M. Taylor by Pasteur's method altered the yellow fever virus by a sequence of passages in white mice (intracerebral infections). After 20-30 passages the strength of the pathogenic activity of the original virus increased upon intracerebral infection. Upon subcutaneous or intranasal infections this altered virus proved to be completely harmless. For a more stable fixation of the acquired properties, it was passed many times through chick embryos. This new variant proved to be quite an effective preparation (live vaccine) in the specific prophylaxis of yellow fever.

N. Gaisky by the method of selection in 1942 obtained live vaccine against tularaemia from the altered tularaemia bacteria as a result of ageing. The vaccine was proved effective and was employed successfully in vaccinating people against tularaemia.

By the method of selection of colonies of noncapsulated microbes and by cultivation on special nutrient media, N. Stamatin, M. Stern, N. Ginsburg and A. Tamarin in 1936-1940 obtained a noncapsular variant of the anthrax bacillus, which at present is successfully used for specific prophylaxis. The Ginsburg and Tamarin vaccine named STI (Sanitary Technical Institute) proved to be more effective than the Pasteur and Tsenkovsky vaccines. It is used for the vaccination of humans and agricultural animals.

It has been established that changes in biological properties may be observed in viruses. In particular, in the influenza virus under the influence of acquired immunity, the structure of the haemagglutinin enzyme changes quite rapidly. The appearance of a new character in the influenza virus is characterized by the increase of its virulence, and the development of epidemic outbreaks of influenza, as in the pandemic in 1957 caused by a new variety—the influenza A2 virus.

Viruses, like bacteria, under the influence of different factors are able to lose their pathogenicity completely or partly, and retain their immunogenic properties. Modern methods of preparing vaccine strains against a number of viral diseases are based on this principle.

At present vaccine strains are employed, which have been obtained from live, but attenuated viruses of poliomyelitis, parotitis, measles, influenza, typhus rickettsiae, etc. Live vaccines are quite effectively used in veterinary practice against infectious diseases in ani-

mals, such as swine erysipelas, cattle plague, swine plague and catarrhal fever in sheep.

The pathogenic properties in viruses can be weakened under the influence of nitrous acid, hydroxylamine, bromosubstituted bases, by increasing the temperature, decreasing the pH of the medium, by the action of supersonic waves, ultraviolet rays and minimal concentrations of ribonuclease.

It has been suggested that the weakening of the pathogenicity of some microbes takes place as a result of the changes in the native properties of DNA or RNA. The deamination of certain nucleotides, depuration, replacement of nitrous bases, cleavage of nucleotides and other actions can bring about the loss of pathogenic properties of viruses while retaining their ability for multiplication.

Numerous scientists have given indisputable proof that microorganisms acquire drug-resistance, become resistant to chemical substances, phages and antibiotics, while the acquired properties are retained for a long time in the following generations.

The mechanism of acquiring drug-resistance by bacteria is explained in more than one way. It is considered that resistance to drugs is acquired as a result of selection and that the properties of stability found in the bacterial genes determine the outcome. Stable ones survive and sensitive ones die. The investigations of microbiologists adhering to another conception have illustrated that the acquisition of drug-resistance takes place under the influence of changes in environmental conditions. The environment as a result of continual changes in its composition provides for the corresponding changes in metabolism, and the selection of individuals which are best adapted to the new conditions. The manifestation of variation consists not only in the selection which takes place due to the original characters in the genes, but as a result of mutations and recombinations in the gene apparatus in response to the changed environment. These kinds of changes are stable and are transmitted to the following generations.

Bacteria are capable of acquiring resistance to ionizing radiations, the mechanism of which is similar to that of drug-resistance. By applying radioactive substances widely differing variants were obtained which are of great practical importance, especially in the manufacture of antibiotics. The altered microorganisms, producers of antibiotics, give a greater yield of active substances than the original forms.

FORMS OF VARIATION IN MICROBES

1. *Intraspecies nonhereditary variation.* This kind of variation is found quite frequently. It occurs under the influence of various comparatively mild effects of the environment on microbes due to

which the ensuing changes are not fixed in a hereditary sense. For example, strains of colibacilli which grow on agar with sodium ricinoleate form long filaments. Upon the addition of calcium chloride these cells become quite short. A deficiency of calcium in the medium provokes an increase in spore production and a slimy growth in anthrax bacilli. Inorganic iron has a great influence on the formation of toxins. A decrease in oxygen lowers the degree of pigmentation and increases the number of smooth colonies in tubercle bacilli. The addition of lithium to the nutrient medium induces growth of microbes in the shape of curious branched giant forms, spheres and finest filaments. Glycerin and alanine induce pleomorphism in cholera vibrios. Microbes can temporarily change their enzymatic (biochemical) ability.

It has been suggested that avisual forms are examples of nonhereditary intraspecies selection. A. Fontès, P. Odurois, V. Suknev, V. Timakov, G. Kalina, R. Tulian, etc., have proved that many microbes are capable of transforming into filterable forms which are characterized by being stable to the action of physical and chemical factors, and do not grow under normal conditions of cultivation.

V. Suknev and G. Wolferts devised a method of feeders with the aid of which they succeeded in transforming filterable forms into bacterial forms. The material under test containing filterable forms was transferred to a dish containing a nutrient medium. Then sarcinae or staphylococci were seeded in separate areas on the medium. In a few days of growth the macrocolonies of these organisms were surrounded by small colonies of bacteria originating from filterable forms.

The production of filterable forms in bacteria is acknowledged. It has been proved by a large number of investigations by Soviet and foreign scientists. This process occurs mainly under the influence of unfavourable conditions: the action of antibiotics, immune sera, phages, low temperatures, chemicals, etc. As a result, changes occur and the cell structure of bacteria is impaired. Filterable forms can be produced also as a result of the physiological ageing of microbial cells. Some filterable forms upon falling into favourable conditions revert to the original form, while others acquire heritable altered properties. The so-called G-forms are produced by regeneration from the filterable forms.

Under the influence of unfavourable conditions of life some species of bacteria undergo profound changes with the formation of peculiar small colonies (L-colonies) with a dark dense centre and a loose edge. These altered microorganisms are known as L-forms of bacteria. They occur, for example, under the effect of penicillin, immune serum, phage, chemicals, irradiation and other unfavourable factors on the culture (see p. 44). The microbial cell changes into a large sphere. Vacuoles, small and large granules appear in it from which L-colonies are formed during reproduction.

Mycoplasmas produce filterable forms with great constancy. They were formerly improperly regarded as viruses.

2. *Intraspecies hereditary variation.* Hereditary variations have been described under different names: mutations, transformations, lysogenic conversions, transductions, conjugations, etc.

A mutation is that process in which new properties associated with changes in the gene apparatus are suddenly acquired.

Mutations are accompanied by changes in the composition or arrangement of the nitrogen bases in definite areas of the DNA molecule. New properties acquired by the microorganism as a result of changes in the gene apparatus under the influence of environmental factors are passed on to the offspring in an unlimited number of populations.

Mutations, as a rule, occur under the influence of strong-acting substances, e.g., toxins, X-rays and other factors.

Darwin's theory of evolution had a definite influence on the development of genetics, and without it, it would have been impossible to comprehend properly the numerous new data which have been obtained over the past 10-15 years. Due to a scientific approach to the problems of genetics and from the Darwinian theory, hereditary variation is regarded as one of the factors in the evolutionary process. Mutations, no matter how chance they may be, are undoubtedly causal phenomena, and can be controlled, since different external agents which provoke mutations have a known mutagenic specificity, although they cannot be considered as factors adequate to the adaptive evolutionary process. When applied to microbiology, variations due to mutations in some cases have quite an adequate specific adaptive nature, although they are spontaneous and casual. Thus, the controversy between the mutation theory and the theory of the transmission of acquired characters in heredity loses its meaning.

A certain tie between the mutation process and evolutionary development was established by I. Schmalgausen (1936-1946), the essence of which is that mutations are the source of disintegrations in the formative processes of the developing young organism. With the onset of mutations, the original genetic system undergoes changes. Together with mutations, disturbances occur in the correlation ties between the parts and organs of the body, which lead to disturbances in the formative processes, to lowering of viability, and to disintegration processes, that is, to the loss of those forms of organization, and that level of integrity which were attained in the previous period of development of the species. This shows the relative harm of mutations due to their disintegrating manifestations, especially in the origin of large defects.

In the process of evolution under the influence of different factors and especially defense mechanisms of the bodies of animals and man, microorganisms undergo essential changes which consist in

the alteration of the ability to react with antibodies of convalescent human and animal sera. These changes are known as type changes, since they do not extend beyond the limits of the species. Type characters are found in many pathogenic species. Thus, for example, meningococcus has A, B, C, and D types, pneumococcus has 80 types and dysentery bacteria—more than 30 types.

Before 1945, influenza was caused by A and B viruses. In 1945 the first outbreaks of influenza caused by the A1 virus occurred. In 1957 the A2 type (Asia 57) occurred which was the causative agent of the pandemic of influenza which seized the whole world.

Intraspecies hereditary variation includes recombinants produced as a result of transformation, transduction and conjugation (see below).

3. *Species-forming variation*. Species-forming variation is a complex process in which the change of any character under the influence of the environment involves a correlated change in the whole complex of metabolic reactions of the whole body. One can imagine that parasites evolved from saprophytes as a result of their adaptation to new conditions of life in the animal organism. Thus, probably the formation of tubercle bacilli, rickettsiae, salmonellae and other causative agents of infectious diseases took place as these organisms gradually adapted themselves to animals and man.

The great majority of pathogenic species for man have precursors. The evidence is the existence of microbe-duplicates (diphtheria bacilli and diphtheroids, pathogenic bacteria and saprophytes, cholera and cholera-like vibriones, *Entamoeba histolytica* and *Entamoeba coli*, anthrax bacilli and anthracoids, pathogenic and nonpathogenic *E. coli*, etc.).

The formation of species is possibly due to mutations occurring under the influence of changes in the genetic material (DNA and RNA, purine and pyrimidine bases).

Mutations can be induced by nucleotide substitution in the nucleic acid, and also by changing the sequence of nucleotides.

THE MECHANISMS CAUSING THE INTRASPECIES AND SPECIES-FORMING VARIATION

Hereditary variation in bacteria is performed with the aid of genetic structures. Bacteria in contrast to plants and animals are haploid organisms. They contain one genome and combine the function of a gamete and an individual. Viruses also pertain to typical haploid living systems. In many bacteria only one chromosome has been found in which the haploid set of genes is enclosed in a certain sequence.

The bacterial chromosome consists of structural elements of the cell nucleus, which have a relatively long length controlling the

complex reaction of the synthesis of some metabolite—amino acid, vitamin, enzyme, nitrogenous base, etc. Each area of this kind is known as a genetic region which includes separate genetic determinants controlling the biosynthesis of intermediate products essential for carrying out the ultimate reaction, i.e., the synthesis of the given metabolite. These subunits are known as genetic loci or simply loci or cistrons. Most scientists designate cistrons as genes.

A gene is the area of DNA in which the sequence of amino acids in the polypeptide chain controlling a particular property of an individual is coded. The unit of mutation is *the muton* which in size is the smallest part of the chromosome (DNA) consisting of one pair of nucleotides, the alterations of which lead to a mutation. *Recon* is the minimal area of DNA which contains one or several pairs of nucleotides and which is capable of recombination. The group of bases with the help of which an amino acid is coded is known as *a codon.*

Hereditary variations in bacteria are subdivided into mutations and recombinations (see transformation, lysogenic conversion, transduction, conjugation).

MUTATION

A mutation is a stable hereditary variation in the properties of the microorganism (morphological, cultural, biochemical, biological, etc.) which is not associated with the process of recombination. There are (1) nuclear mutations, i.e., hereditary variations which take place in the nucleus and are passed on by certain segments (nucleotides) of the molecule of DNA; (2) cytoplasmic mutations, i.e., hereditary variations which occur in the cytoplasm and are passed on by the cytoplasmic structures.

Mutations are mainly accompanied by the falling out (deletion) or the addition (duplication) of one or a small group of bases in the DNA molecule (Fig. 54). A whole series of characters may be exposed to mutations—auxotrophy of amino acids, purines, pyrimidines, vitamins, sensitivity or resistance to antibiotics, sulphonamides, phages, enzymatic activity, etc.

Bacterial mutations can be divided into: (a) spontaneous which occur under the influence of external factors without the interference of the experimenter and (b) induced which occur due to treatment of the microbe population with mutagenic agents (radiation, temperature, chemical and other effects, etc.).

There are large and small (point) mutations. Large reorganizations include mutations which are characterized by the falling out of a large part of the gene. A point mutation occurs inside the gene itself, and represents a change only in one pair of the nucleotides. Large mutations are accompanied by the cleavage of the polynu-

cleotide chains which leads to the disintegration of all the systems of the bacterial cell. During point mutations spontaneous and induced reversions may take place which lead to the renewal of the lost property, while a large mutation does not bring about direct reversible mutations.

Frequently among microorganisms the renewal of prototrophy of biochemical mutants is observed as a result of changes in the regions of genetic material unharmed by direct mutation. These kinds of changes known as suppressor mutations may occur within the limits of the mutant gene as well as beyond the limits. Suppressor mutations have a wide range of action.

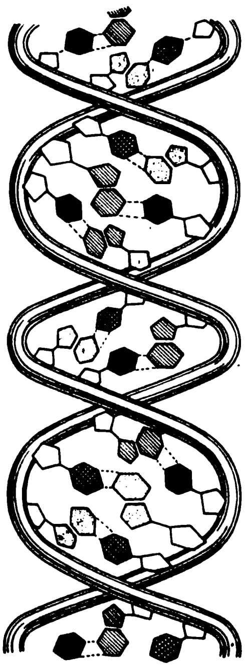


Fig. 54. Diagram of the structure of DNA

To reveal mutants different methods are used. If the microbe population exposed to the action of mutagens differs in cultural properties, then these mutagens can be differentiated in size, shape, structure and colour of colonies. Mutations of biochemical properties can be detected with the help of minimal media which contain only salt and carbohydrates. Prototrophs can grow in a minimal medium, as they are capable of synthesizing the necessary metabolites for their development (e.g., amino acids, vitamins, nucleic acids, etc.), while auxotrophs require certain media containing the essential metabolites. During biochemical mutations a transition occurs from the prototrophic to the auxotrophic type of nutrition.

For the purpose of isolating mutant cells the method of replica plating is employed. The diameter of the replica is the same as that of a Petri dish. Velvet is stretched over the replica. First, the dish with the colonies under test which are produced on a complete medium is pressed on the replica with the velvet. The cells of separate colonies remain on the nap of the velvet. This replica is applied to two dishes with a minimal and a complete medium. The cultures obtained from these media are identified, and the requirement for one of the metabolites is established.

To reveal reversible biochemical mutations from the auxotrophic to the prototrophic type of nutrition, the method of selective media is employed where cells of a definite genotype grow. If *E. coli* which requires biotin is seeded on a medium devoid of it, then the mutants can be selected which renew the ability to synthesize biotin. Thus,

a medium devoid of biotin is unsuitable for the growth of cells obtained as a result of direct mutations and is selective for revealing reversible mutations. By using selective media, mutants are revealed which are resistant to antibacterial preparations. For this, the seeding of the culture under test containing some antibiotics (penicillin, streptomycin, etc.) is carried out. All of the cells which are sensitive to this preparation perish, while the resistant ones will survive, and as a result of their growth, colonies are formed. With the help of selective media not only mutations can be detected, but also adaptations in bacteria in the formation of which other mechanisms take part (see p. 157). At present there are a series of investigations being carried out confirming the mutagenic nature of the adaptations of microorganisms.

RECOMBINATION

Transformation. In 1928 F. Griffits established that upon infecting mice with small doses of a nonpathogenic decapsulated type II pneumococcus culture, together with pathogenic capsulated type III culture killed by heating, profound changes occur in the type II pneumococci. They acquire virulent properties and a capsule typical of type III pneumococcus.

At present the mechanism of this kind of variation has been elucidated, and has been given the name of **transformation**. In 1944 O. Avery, C. McLeod and M. McCarthy subjected a type III pneumococcus culture, which had been previously heated to a temperature of 65°C for 30 minutes, to the action of sodium desoxycholate. The extract was precipitated by alcohol and then treated with chloroform. A substance was obtained with a high viscosity, a very slight amount of which brought about the transition of any type of pneumococcus culture to type III pneumococcus. This substance was found to be desoxyribonucleic acid.

In later investigations it was established that DNA has a transforming action not only on type specificity, but on other characters (phage lysability, resistance to antibiotics, etc.).

DNA has been used as a transforming agent and for causing changes in other species of microbes (*Mycobacterium tuberculosis avium*, haemoglobinophilic bacteria of influenza, meningococcus and Flexner's shigella). The transforming activity of DNA is very high. Its contact with the changing culture for 10-15 minutes is quite sufficient to provoke the beginning of variation which is completed in two hours.

Analysis of the structure of DNA has shown that there is a definite relation between two definite bases—adenine and thymine, their amount in any molecule of DNA is the same (A=T), while the amount of guanine is equal to the amount of cytosine (G=C). It was revealed

that in the DNA of each species there is a definite ratio $\frac{A+T}{G+C}$. Later it was shown that this pairing of bases is associated with the fact that the DNA has a double strand structure, and both strands (polynucleotides) are bound together by bases which in turn are bound by hydrogen. The sequence of nucleotides in the polynucleotide filament of DNA can vary, but the nitrogenous bases on the same or different chain are complementary and always paired. The adenine of one chain is always opposite to thymine in the other, and guanine opposite to cytosine. These investigations had a great influence on elucidating very important problems in genetic information.

For the synthesis of proteins, the bacterial cell must obtain the necessary amount of amino acids which can be governed by a three letter code, and with the help of which all amino acids making up the protein are coded. The amount of possible combinations of nitrogenous bases reaches more than two million, and reflects the number of species of organisms populating our planet.

Some scientists regard transformation as a process of selection during which only a small part of the cell is exposed to variations. Under the influence of high temperature, ultraviolet and X-rays, desoxyribonuclease, and chemical mutagens, the transforming ability of DNA is lowered considerably during which the number of transformed elements is also decreased. The treatment of transforming DNA with nitric acid is accompanied by a rapid loss of the transforming activity of the preparation without a change in the viscosity of the desoxyribonucleic acid. Heating at 80-100°C also leads to profound changes in DNA as a result of the cleavage of hydrogen bonds between the coils of the double DNA spiral with the formation of single coils structures. A subsequent slow cooling promotes the reformation of the double spiral.

It is of considerable importance to ascertain those factors which help to increase the frequency of transformation. So far there are no data on this problem apart from separate investigations concerning the hay bacillus and pneumococcus. The frequency of transformation of the hay bacillus can be increased by adding inorganic phosphate to the medium. The introduction of albumin into the medium with the growing recipient culture of pneumococcus also increases the number of transformed elements. The optimal temperature during the period of contact of DNA and the cell is considered to be 29-32°C.

Thus, the effectiveness of transformation depends on a whole series of conditions (the composition of the medium, temperature), the physiological state of the recipients and the transforming DNA (polymerism, retaining of the double spiral). The frequency of transformation varies within 0.047-0.0004 per cent.

Of theoretical as well as of practical importance is the possibility of transformation among microorganisms of different species. As

already observed, the appearance of recombinants is preceded by the recombination of the transforming DNA donor with the genome of the recipient cell. It depends on the size of the genetic marker (linked gene which controls the synthesis of amino acids, carbohydrate fermentation, resistance to antibacterial preparations, etc.). The greater the size of the marker, the less the frequency of incorporation by the genome of the microbial cell. In the transformation of two closely linked genetic markers situated on one molecule of DNA, the transformed elements with single characters appear more often than those transformed elements which bear two characters simultaneously. Besides, the frequency of incorporating the marker depends on the structure of the adjacent parts of the DNA molecule. Figure 55 shows that the homologous parts in the process of transformation are arranged parallel to one another, and are in close contact. In the absence of the corresponding homology of the DNA donor and recipient, the process of recombination is impaired, which

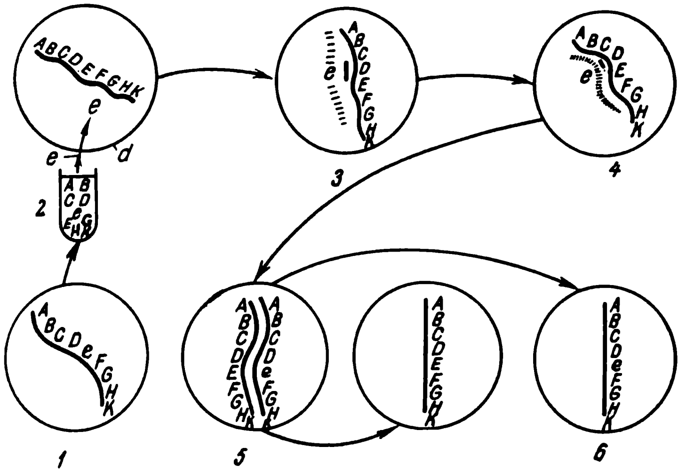


Fig. 55. Mechanism of transformation

1—cell with genotype ABCDeFGHK; 2—the penetration of the DNA of cell "1" into the cell with the genotype ABCDEFGHK; 3,4—origin of cells with unlike chromosomes; 5—diploid cell, having two different chromosomes; 6—formation of a new cell with the genotype inherent in cell "1"

leads to a marked decrease in the frequency of the incorporation of the genetic material in the genome of the bacterial cell. Thus, the interspecies transformation is possible only if the DNA donor and recipient are very similar in nucleotide composition.

The transforming agent upon its incorporation into the genetic apparatus of the recipient cell fulfils the role of a hereditary factor analogous to the gene.

During transformation transmission is made possible not only in independent (unlinked), but in linked characters also. If a strain of pneumococcus which is resistant to streptomycin and can ferment mannitol is taken as the donor, then it is possible to receive transformed elements bearing both characters, with a frequency greater than the possibility of the two independent characters coinciding. Upon the addition of a mixture of DNA isolated from two strains bearing each of these characters separately to a recipient culture, such a number of double transformed elements will not be observed.

Transformation is that process with the help of which one character is substituted by another. Thus, for example, one can experimentally reproduce resistance to an antibiotic, and vice versa, make the resistant strain sensitive. Consequently, in the process of transformation we observe not simply the addition of a new character, but the substitution of one genetic factor by another. This phenomenon is similar in nature to the gene manifestation in the chromosomes of plant and animal cells.

The discovery of transformation allows us to study more profoundly the genetic role of DNA at the molecular level, and to elucidate a whole series of problems concerning microbial associations, as to variations, infectious processes and immunity.

Lysogenic conversion. In 1955 N. Groman and M. Eaton, and later other investigators established that nonlysogenic and nontoxigenic strains of diphtheria bacilli as a result of lysogenicity transform into toxigenic strains. It has been suggested that not only the prophage of lysogenic bacteria takes part in the mechanism of lysogenicity but also definite systems of the bacterial cell, as a result of which changes occur in the biosynthetic processes.

The presence of the prophage in bacterial cells may not only provide for the change in toxigenicity, but also in other properties (morphology of the colonies, synthesis of new antigens in salmonellae, the ability to ferment carbohydrates, etc.). The variation produced under the influence of a prophage is known as *lysogenic conversion*, which occurs due to lysogenicity of bacteria. Consequently, lysogenicity is one means of acquiring hereditary characters, while moderate phages producing lysogenicity are one of the factors which are capable of carrying the genetic bacterial characters.

Some investigators have associated toxin production with the lysogenicity of the culture. Later it appeared that no direct relationship between lysogenicity and toxigenicity had been established. Many strains of diphtheria bacilli may be lysogenic and nontoxigenic and vice versa, toxigenic cultures are not always lysogenic.

Lysogenic conversion in natural conditions is of great significance not only for the origin of toxigenic strains of diphtheria bacilli, but also of antigenic variants of salmonella. It is possible, due to lysogenic conversion, that a comparatively more intensive formation

of bacterial types of this species occurs, the number of which at present exceeds 700.

Lysogenic bacteria are the most convenient biological models for the study of a whole series of problems: the relationship of the virus and cell, the nature of immunity, biological effectiveness of ionizing radiation, elaboration of methods for obtaining antiviral and antitumoral medicinal substances, etc.

Genetic transduction. Changes known as **transduction** have been found to occur in bacteria (N. Cinder and J. Lederberg, 1952), during which the phage carries the hereditary material from the donor bacteria to the recipient bacteria. Thus, for example, with the help of the phage the transduction of flagella, enzymatic properties, resistance to antibiotics, virulence and other characters can be reproduced. The donor bacteria, the phage transducer and recipient bacteria (Fig. 56) take part in the phenomenon of transduction.

The mechanism of transduction consists of the following stages. In the process of multiplication of certain temperate phages small fragments of genetic material (DNA) of bacteria enter a particle of a newly formed phage. Upon infection of a recipient bacteria with such a phage fragments of the genetic substance of the donor bacte-

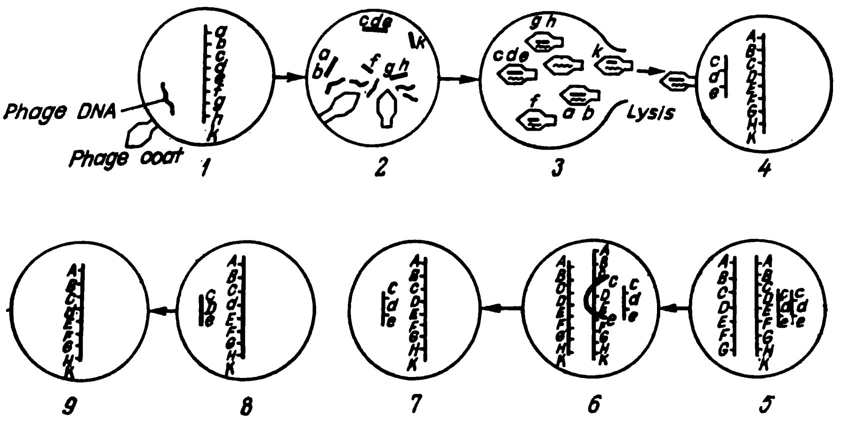


Fig. 56. Mechanism of transduction

1—cell with the genotype abcdefghk, into which the phage DNA has penetrated; *2*—breakdown of bacterial chromosomes and the formation of phage particles; *3*—seizure of pieces of bacterial chromosomes by phage particles, lysis of the bacterial cell; *4*—penetration of the phage—chromosome c,d,e particle complex; *5*—autoreproduction of own chromosome in the bacterial cell and a chromosomal fragment from another cell, introduced by the phage; *6*—double crossing between the chromosome and the homologous fragment of the foreign chromosome; *7*—a cell with a non-crossing-over chromosome and a fragment of a foreign chromosome; *8*—cell with a crossing-over chromosome and a fragment of a foreign chromosome; *9*—a cell the chromosomes of which received a gene "d" under the influence of genetic material from a fragment of a foreign chromosome, carried in by the phage (transduction)

ria may recombine with the genetic material of the recipient bacteria. Various hereditary characters are usually transduced independent of one another.

There are two types of transduction—general and special. During general transduction recombination of any character or several characters may occur. The frequency of transduction of the general type is within the limits of 10^{-4}-10^{-7} per phage particle. Transduction of a definite character may occur independently of other characters, if the latter are not associated with the transduced character. The frequency of general transduction can be increased by preliminary treatment of the transducer phage particles with ultraviolet irradiation.

A special type of transduction is carried out only by the phage, obtained as a result of induction of lysogenic strains of bacteria by ultraviolet irradiation. During special transduction the closely bound group of characters which control the utilization of galactose (galactose locus) is transmitted, and while such characters as the utilization of carbohydrates, the ability to synthesize amino acids, and sensitivity to antibiotics, etc., are not transmitted. Special transduction is observed in all cells independent of their sensitivity to the λ-phage. However its frequency is greater in sensitive cells than in lysogenic or immune cells.

Special transduction is observed when the phage originates from the galactose positive donor bacteria. In this case the λ-phage produced by the galactose positive lysogenic cells transduces the galactose positive character, but not any other character.

Of considerable importance is the possibility of interspecies transduction, since it is rather well known, that the infectious process occurs usually in conditions of mixed infection leading to a reduction or reinforcement of the pathogenicity and toxin production of separate co-members of the parasite coenosis (microflora of the intestine, pharynx, nose, vagina, etc.).

Conjugation. The fusion of the chromatin matter of two related species or types of bacteria and viruses is known as *conjugation* and resembles a reduced sexual process (see Fig. 31). It has been established that the transmission of chromatin particles from the donor cell to the recipient cell occurs in a definite direction and with a known frequency.

In 1946 J. Lederberg and E. Tatum established that during mixed cultivation of two strains of colibacilli differing from each other in respect to several characters, recombinants arise, which are new forms of bacteria bearing some characters of one and some characters of the other original strain.

Upon mixed cultivation of viruses, the phenomenon of conjugation (hybridization) of related species or strains is observed.

At present conjugation is considered to be a one-sided transport of genetic material from one cell to another. A necessary condition

for conjugation is the presence of a specific fertility factor, designated F in one of the conjugating cells.

One of the cells performs the function of a donor, the other—of a recipient. Thus, conjugation has the general characters of transformation and transduction, although it differs essentially from the latter phenomena. The fertility factor in the cytoplasm reduplicates independently of the genetic structure of the nucleus.

Upon treating the cells F^+ with acridine dye, they lose their fertility factor and change into F^- cells. This demonstrates the cytoplasmic nature of the F factor, as acridine dyes do not have any effect on the nuclear apparatus.

Cells which act as donors are designated F^+, and cells which act as recipients —F^-. Crossing of $F^+ \times F^-$ and $F^+ \times F^+$ is accompanied by fertility, while crossing of $F^- \times F^-$, by sterility. It has been suggested that in the cytoplasm of each F^+ cell, there is not one but several particles of this factor.

The penetration of the F factor takes place only during direct contact of the bacterial cells. It has not been possible to isolate it in a free state.

Genetic analysis is constructed by the use of certain methods and the compilation of special charts. Crossed strains of microorganisms differ from one another by a number of characters designated by the first letters of the corresponding word. The ability to synthesize the required metabolites or to ferment certain carbohydrates is indicated by the symbols with the sign (+), and the absence of this ability is indicated by the symbols with the sign (—). The index *s* designates sensitivity, and the index *r*—resistance to antibiotics and phages.

This can be demonstrated by crossing two strains of *E. coli* K12, designated as A and C. The A strain lacks the ability to synthesize biotin (B) and methionine (M). The C strain is unable to synthesize threonine (T), leucine (L) and vitamin B_1. These can be expressed as $T^+L^+B_1^+M^-B^-$(A) and $T^-L^-B_1^-M^+B^+$ (C). If both strains are sensitive (*s*) to the phage (T1) or both are resistant (*r*) then all the recombinants as well as the parent forms will be $T1^s$ (sensitive) in the former and $T1^r$(resistant) in the latter case.

Upon crossing $T^+L^+B_1^+M^-B^-T1^r \times T^-L^-B_1^-M^+B^+T1^s$(1) and $T^+L^+B_1^+M^-B^-T1^s \times T^-L^-B_1^-M^+T1^r$(2), in the first case 75 per cent of the recombinants produced will have the character $T1^r$ of the original type A, and 25 per cent with the character $T1^s$ of the original strain B. In the second case 64 per cent of the recombinants will bear the character $T1^s$ of the A type and 36 per cent—the $T1^r$ character of the B type.

To carry out genetic analysis many more hereditary characters are used (the ability to synthesize important metabolites and ferment a number of carbohydrates designated Lac, Mal, Man, Gal, etc., sensitivity to the phage, antibiotics and toxic substances).

Upon simultaneous cultivation of bacteria of two different mutants in liquid medium, cells possessing characters of both parent types in various combinations were isolated.

Conjugation like other recombinations can occur not only between strains of one and the same species, but among cells of different species. There is no doubt about the interspecies conjugation between *E. coli* and dysentery bacteria, which leads to the formation of the

Type of crossing	Strains being crossed	Result of crossing
$F^- \times F^-$	$B^-M^-F^- \times T^-L^-B_1^-F^-$	Complete sterility
$F^+ \times F^-$	$B^-M^-F^- \times T^-L^-B_1^-F^+$	Very high fertility
	$B^-M^-F^+ \times T^-L^-B_1^-F^-$	
$F^+ \times F^+$	$B^-M^-F^+ \times T^-L^-B_1^-F^+$	High fertility

most varied changes in bacteria known as atypical strains. Of special interest is the question of the origin of pathogenic strains of colibacillus causing colienteritis in children.

The essence of conjugation is interpreted differently: some scientists consider it to be the result of the exchange of genetic structures, while others designate it as vegetative hybridization.

The process of conjugation can be controlled and definite characters can be made to pass from one bacterial cell to the other.

Temperate phages are typical models of episome elements. They are genetic elements, exogenic in relation to the bacterial cell which is able to exist without them. Upon penetrating the bacterial cytoplasm, temperate phages can reproduce vegetatively (autonomously) or may enter an integrated state—prophage. These two forms mutually exclude one another. The autonomous form of existence of the phage leads to bacterial lysis. Upon integration, the genetic material of the phage is localized in the bacterial chromosome. Genetic recombination may occur between the prophage and areas of the bacterial chromosome.

The episomes include *the sexual factor* of bacteria, which is not necessary for the bacterial cell. It occurs in the type F^+ bacteria and is absent in the type F^- cells. This factor may be found in an autonomous state in which it multiplies independently of the bacterial chromosome, and in the integrated state when this factor is localized on the bacterial chromosome, and is reproduced together with it. The transition from the autonomous to the integrated state determines the mutation $F^+ \rightarrow Hfr$ (Hfr—high frequency recombination), while the transition from the integrated to the autonomous state provides for the mutation $Hfr \rightarrow F^+$.

Episomes include *factors of colicinogenesis* which control the synthesis of definite antibiotics of the bacteriocin type. The latter are of a protein nature and give rise to definite strains. They effect other strains of the same species. The best investigated are the colicins which have antibiotic properties in relation to some strains of the colibacillus.

Episomes take part in causing lysogenic conversions. They control the production of toxins in certain strains of *Corynebacterium* organisms, of capsules in pneumococcus and anthrax bacilli, of

pigments in some species of pseudomonas, of spores in bacilli and of flagella in salmonellae.

At present there undoubtedly exists a strict correlation between nucleic acid and the structure of proteins. Nucleic acids play a large part in the phenomenon of heredity and variation in microorganisms. It has been proved that under the influence of ionizing radiation and chemicals, variants of microorganisms were obtained having new properties in comparison to the original strains. The present stage of the development of genetics of microbes permits us to consider that besides the methods of directing heredity by selection, other methods of obtaining mutants with the help of transformation, transduction, lysogenic conversion and conjugation may also be employed.

Currently it has been established that not only the nuclear material of microbial cells has genetic properties, but also the plasmids capable of autonomous multiplication. Episomes are intermediate as they carry out a genetic function within as well as without the nucleus. The episomes of one-step mutations may change to the plasmid state. During recombination the chromosome determinants have episome properties.

At the present stage of development of Soviet microbiology, theoretical points built on a scientific understanding of the problems of natural science are widely used in the practice of laboratory diagnosis of infectious diseases, in preparing vaccines, sera, phages, antibiotics, and also in combatting drug-resistant forms of many causative agents of infectious diseases.

By the method of selection special cultures of yeasts and other microbes have been obtained, which are used in the technology of food products, in the production of anatoxins, vaccines, antibiotics and in industrial microbiology. Thus, for example, at present in the production of penicillin, strains of the mould *Penicillium* give 500-1,000 times greater yields of penicillin per unit volume of cultural fluid, than those strains from which penicillin was first isolated.

Under the influence of defense mechanisms of the human body, and highly effective chemical and biological preparations, and also in conditions of microbe associations pathogenic microorganisms, within a comparatively short time, have undergone considerable changes in morphological, cultural, biochemical and biological properties. Deviations occur in all the characters of the causative agent from the typical, original forms which make it difficult to determine the species of the isolated cultures.

Atypical forms of causative agents are exposed to a detailed investigation with the use of special methods, allowing in a number of cases to reveal hidden (nonheritable) characters and to restore the lost microbial properties. One of these methods of demasking microbes is to grow them on media containing bile.

The preparation of effective vaccines requires, first of all, a selection of such universal strains which would correspond to the most frequently widespread causative agents, and which could stimulate the organism to produce species and type immunity. This kind of prerequisite is made in relation to the preparation of medicinal sera, phages and diagnostic preparations.

Many microbes become adapted to different drugs. In treatment of infectious diseases, the sensitivity of pathogenic microorganisms to the applied medicinal preparations is checked regularly.

It has been established that lysogenic bacteria do not differ from the normal bacteria in morphological, cultural and biochemical properties. The difference is that lysogenic bacteria are quite sensitive to ultraviolet and X-rays, under the influence of which they lose their lysogenicity and become sensitive to the phage. They may be used for studying many problems associated with outer space.

PART TWO

*

THE STUDY OF INFECTION AND IMMUNITY

Translated by V. Lisovskaya

INFECTION AND INFECTIOUS PROCESS

The term infection (Lat. *infectio*—to infect) signifies the sum of biological processes which take place in the macroorganism upon the penetration of pathogenic microorganisms into it, independent of whether the penetration will entail the development of an obvious or a latent disease or whether the macroorganism will only become a temporary carrier of the causative agent.

The historically developed interaction of the susceptible human organism and the pathogenic microorganism in certain conditions of the external and social environment which gives rise to an obvious or latent pathological process is called *an infectious process*.

From the biological point of view, the infectious process is a kind of parasitism in which two live organisms adapted to different environmental effects enter into combat.

Infectious disease designates one of the extreme degrees of manifestation of the infectious process.

Infectious diseases are considered to be phenomena including biological and social factors. Thus, for example, the mechanisms of transmitting infectious diseases, their severity and outcome are provided for mainly by social conditions.

Infectious diseases differ from other diseases in that they are caused by live causative agents of a plant and animal origin and are characterized by contagiousness, the presence of a latent period, specific reactions of the body to the causative agent and production of immunity.

Due to the development of genetics of viruses at the present time, the conception of the infectious agent has been considerably extended. During many viral diseases (tobacco mosaic disease, influenza, foot-and-mouth disease, tick-borne encephalitis, diseases caused by adenoviruses, some tumours, etc.) high molecular structures DNA or RNA possess infectious properties. DNA and RNA are not organisms, but are capable of bringing about the genetic information inherent in the corresponding viruses. Consequently, besides diseases in which the infectious process is caused by live substances,

the existence of molecular infections has been confirmed (see p. 183). It has been suggested that molecular infections are characterized by the ability to be transmitted not only through the external environment, but from the parents to the offspring.

The origin of pathogenic microbes and infectious diseases goes back to ancient times. As a result of a long-term evolutionary process, parasitic species of microorganisms were formed, capable of causing various infectious diseases in humans under certain conditions.

It is considered that cocci are the most ancient microorganisms. They have been found in limestones of the Proterozoic Era and in coal rock of the Paleozoic Era.

In the process of evolution some species of coccal forms acquired the ability of a parasitic way of life. The appearance of pathogenic cocci is believed to date from the Permian period. In the deposits of this period of the Earth great changes were discovered in the bones of reptiles. It is possible that some of these changes were the consequence of diseases caused by infectious species of cocci.

It is more likely that from the aquatic saprophytic and free-living vibrios, pathogenic vibrios were formed. Between the aquatic and cholera vibrios there are intermediate (paracholera) forms (*Vibrio El Tor*, *Kutcher*, *Finkleri-Prior* and *Metchnikovii*, etc.).

The origin of *Mycobacterium tuberculosis* also can be traced back to ancient times. Over a long period, tuberculosis as well as its causative agents has undergone a considerable evolution. The fact that the known species and variants of *Mycobacterium* are parasites of different animals not closely related to each other—warm-blooded (birds, rodents, cattle, humans) and cold-blooded (fishes, snakes, turtles, frogs)—suggests the antiquity of mycobacteria.

Pathogenic microorganisms could have originated due to the adaptation to the human body of organisms parasitic on domestic and synanthropic (living near the homes of man) animals (causative agents of typhus fever, enteric fever, smallpox) or on wild animals (causative agents of relapsing fever, yellow fever, skin leishmaniases).

FORMS OF SYMBIOSIS

According to the character of interrelationship with the plant and animal world, microbes can be subdivided into two groups: saprophytes and parasites.

Saprophytes include microorganisms unable to cause disease.

Parasites are microbes which live at the expense of plant and animal bodies.

All kinds of associations of the macroorganisms and microorganisms constitute symbiosis in its broadest sense. Symbiosis has different forms: commensalism, mutualism and parasitism.

Commensalism is a kind of symbiosis (association) of organisms in which one of them lives at the expense of the other without causing it any harm. The overwhelming majority of representatives of the normal microflora of the human body belong to microbe-commensals.

Mutualism is that kind of symbiosis in which both organisms concerned receive mutual benefit from their association. For example, the symbiosis of nodule bacteria with legume plants is characterized by typical mutualism. Nodule bacteria live in plant roots, while the legumes for their nutrition utilize nitrogenous compounds produced by the bacteria from atmospheric nitrogen.

Some species of bacteria from the group of intestinal microflora live in symbiosis with animal organisms which they inhabit. These microbe-mutualists feed on food remains which enter the lower part of the intestine, while the vitamins which they produce are used by the animals for biocatalytic reactions.

Parasitism is that state of symbiosis in which one organism (parasite) lives at the expense of another (host) and is harmful to it. Many microbe-parasites are capable of causing infectious diseases in plants and animals.

Disease-producing species of microorganisms are known as pathogenic organisms. They have adapted themselves in the process of evolutionary development to a parasitic type of nutrition in tissues and fluids of the animal body. The susceptible infected organism responds to the entry of the pathogenic microbe by nonspecific and specific biological reactions. These are expressed in atypical or typical manifestations of the disease, and also in a variety of defense adaptations.

At one time J. Henle and then R. Koch (1878, 1882) formulated three conditions in the presence of which the given microbe can be recognized as a causative agent of a disease. Henle-Koch's triad consists in the following: (1) the microbe-causative agent should be discovered in all cases during a given disease, and is found neither in healthy persons nor in patients with other diseases; (2) the microbe-causative agent should be isolated from the patient's body in a pure culture; (3) the pure culture of the isolated microbe should cause the same disease in susceptible animals. At present this triad has lost its significance to a considerable degree.

For the origination and development of the infectious process three conditions are necessary: (1) the presence of a pathogenic microbe, (2) its penetration into a susceptible macroorganism, and (3) certain environmental conditions in which the interaction between the microorganism and macroorganism takes place.

The interrelationship of the pathogenic microorganism and the susceptible macroorganism takes place under the complex conditions of the **parasite coenosis**, that is, in various relationships with other microbes and protozoa.

The results of the penetration of pathogenic microbes into the human body depend not only on the reactivity of the macroorganism, but on the normal microflora of the human body, which can express itself antagonistically as well as synergistically.

Besides pathogenic organisms, there is a comparatively large group of microorganisms known as conditionally pathogenic microorganisms living on the skin, in the intestine, in the respiratory tract and uro-genital organs. In normal physiological conditions of life, conditionally pathogenic microbes do not cause disease, that is, they are saprophytes, but during overstrain, overheating, cooling, intoxication and ionizing irradiation of the host body they become capable of causing a series of diseases—autoinfections (see p. 196).

MAIN FEATURES OF PATHOGENIC MICROORGANISMS

Pathogenicity. This is the potential capacity of certain species of microbes to cause an infectious process. Pathogenicity is characterized by a complex of pathogenic properties in the microbe formed in the process of the historical development of the struggle for existence and adaptation to parasitic life in plant, animal and human organisms. Pathogenicity is a specific character of pathogenic microbes.

Pathogenic microbes as stimulants, for the most part, are characterized by a specific action. Each species is capable of giving rise to a definite infectious process.

The specificity of the infectious process is quite an important feature which becomes evident in the localization of the causative agent, selectivity of tissue and organ affection, clinical picture of the disease, mechanisms of isolating microbes from the organism and in production of immunity. The peculiarities of each causative agent as an extreme stimulant are taken into account when devising methods of clinical and laboratory diagnosis, of therapy and prevention of infectious diseases.

Historically developed ecological factors play an essential role in the development of the specificity of pathogenic microorganisms and their ability to cause diseases in certain species of hosts. These factors ensure a definite and regular nature of the transmission of the causative agent from one individual to another.

Virulence. Virulence signifies the degree of pathogenicity of the given culture (strain). Virulence, therefore, is an index of the qualitative individual nature of the pathogenic microorganism.

Virulence in pathogenic microbes changes under the influence of natural conditions.

It can be increased by a sequence of passages through susceptible laboratory animals, and also by transformation, transduction and lysogenic conversion (see "Genetics of Microorganisms").

Virulence can be weakened by the action of different factors on the microorganism, e.g., the defense forces of the organism, antimicrobial preparations, high temperatures, immune sera, disinfectants, seeding from one nutrient medium to another, etc. Artificial reduction of the virulence of pathogenic microbes is widely used in the preparation of live vaccines, applied for the specific prophylaxis of a number of infectious diseases.

In characterizing pathogenic microbes a unit of virulence has been established —Dlm (Dosis letalis minima), representing the minimum amount of live microbes which in a certain period of time bring about death of the corresponding laboratory animals. Since animals have an individual sensitivity to a pathogenic microbe, then the minimum lethal dose Dcl (Dosis certa letalis) which will kill 100 per cent of the experimental animals has been established. This provides for a more accurate characteristic. At present Dl_{50} (the dose which is lethal to one half of the infected animals) is considered to be the most suitable, the use of which allows for a minimal correction in evaluating the virulence in pathogenic bacteria, and may serve as an objective criterion for comparison with other units of virulence.

That number of pathogenic bacteria which is capable of giving rise to an infectious disease is known as the infectious dose of a pathogenic microorganism.

The action of small and large doses of microbes is of great significance in the development of the infectious process, in the length of the incubation (latent) period, and in the severity and outcome of the disease.

Under favourable conditions one microbial cell with a cell division rate of 20 minutes can give a progeny of 250,000 individuals in six hours, and in several hours the amount of microbes may attain many thousand millions which create a large physiological burden on the tissues and organs of the infected organism.

The virulence of pathogenic microorganisms is associated with toxin production, invasiveness, capsule production, agressiveness and other factors.

Microbial toxins. According to the nature of production, microbial toxins are subdivided into exotoxins and endotoxins. Exotoxins include toxins produced by the causative agents of botulism, tetanus, gas gangrene, diphtheria, and by some species of *Shigella* and haemolytic streptococci.

Exotoxins easily diffuse from the cell into the surrounding nutrient medium. They are characterized by a markedly distinct toxicity, and act on the susceptible organism in very small doses. Exotoxins have the properties of enzymes hydrolysing vitally important components of the cells of tissues and organs.

A certain latent period elapses from the moment of injection of the exotoxins into the animal body to the onset of the disease, which

varies from several minutes (toxins of staphylococci and *Clostridium septicum*) to several hours and days (botulism toxin). Exotoxins have a selective pharmacological action infecting separate organs and tissues of the body.

The diphtheria toxin causes necrosis of the tissues at the site of injection, and injures the adrenals and cardiac muscle, while tetanus toxin affects the motor nerve cells, etc.

On parenteral injection of exotoxins into the body, they cause the production of specific substances (antibodies) in the blood, capable of neutralizing these toxins.

In chemical structure exotoxins belong to substances of a protein nature. They are weakly stable to the action of light, oxygen and temperature (they decompose at 60-80°C within 10-60 minutes, and on boiling they break down immediately). In a dried condition they are more stable to high temperature, light and oxygen. The addition of saccharose to the toxins also increases their resistance to heat. Under the influence of 0.3-0.4 per cent formalin and 38-40°C temperature, diphtheria toxin within 30 days loses its toxic properties and changes into an anatoxin.

Some exotoxins (diphtheria, tetanus and gas gangrene) break down under the influence of digestive enzymes as a result of which they become harmless when administered orally. Other exotoxins (of *Clostridium botulinum*, *Clostridium perfringens* and pathogenic staphylococci) do not break down in the stomach and intestine and cause intoxication of the organism during oral administration.

The potency of toxins is determined on sensitive laboratory animals according to Dlm and Dl_{50}. For example, 1 Dlm of the diphtheria toxin represents the minimal amount which during subcutaneous injection into 250 g guinea pigs kills them on the fourth day.

The minimal lethal dose of the native diphtheria toxin for the guinea pig is within the range of 0.002 ml, tetanus toxin for white mouse—0.000005 ml, and botulinus toxin for the guinea pig—from 0.00001 to 0.000001 ml.

In recent years pure tetanus, botulism and diphtheria toxins have been obtained. They are purified by different methods: coagulation at the isoelectric point, repeated precipitation by trichloroacetic acid at a low temperature and a pH of 4.0, salting out by ammonium sulphate and adsorption by various substances.

Purified toxins have a characteristically high toxicity for sensitive laboratory animals. Thus, for example, 1 mg of the diphtheria toxin contains 40,000,000 Dlm for the guinea pig, and 1 mg of botulinus toxin contains 1,000,000,000 Dlm for the white mouse. Crystalline toxins are even more toxic.

The causative agents of enteric fever, paratyphoids, dysentery, gonorrhoea, meningitis and many other Gram-negative bacteria do not produce exotoxins, they contain endotoxins. Endotoxins are

more firmly bound with the body of the bacterial cell, are less toxic and act on the organism in large doses; their latent period is estimated in hours usually, and the selective action is poorly expressed. According to chemical structure endotoxins are related to glucoside-lipid and polysaccharide compounds or phospholipid-protein complexes. They are thermostable. Some endotoxins withstand boiling and autoclaving at 120°C for 30 minutes. Under the effect of formalin and a high temperature they are partially rendered harmless (Table 9).

Table 9

Comparative Characteristics of Toxins

Exotoxins	Endotoxins
Composed of proteins, have the properties of enzymes, some have been obtained in a crystalline state	Composed of glucide lipoprotein complexes, glucide-lipid compounds and polysaccharide specific complexes
Easily diffuse from the cell into the surrounding medium	Firmly bound within the bacterial cell
Highly toxic, characterized by the selective affection of certain organs and tissues	Less toxic, selective action poorly expressed
Thermolabile	Thermostable
During parenteral injection cause the production of highly active antibodies (see p. 225, 232)—antitoxins	During parenteral injection produce precipitins, lysins, opsonins, agglutinins and complement-fixing antibodies
Under the influence of 0.3-0.4 per cent formalin and a temperature of 38-40°C change to anatoxin	Under the influence of formalin and temperature are partially rendered harmless

According to chemical composition toxins can be divided into protein, glucide-lipid and polysaccharide.

Toxins of a protein nature were obtained at first from plants (ricin from castor beans, robin from acacia bark) and from animals (snake venom).

Protein toxins can be divided into three groups according to the nature of interrelationship with the cells which produce them.

1. Toxins found in the nutrient medium, and, with the help of filtration, separable from the bacterial cells. These toxins are named exotoxins, since they are excreted by cells into the surrounding nutrient medium.

2. Toxins more closely bound with the bacterial cells and obtained by extraction with weak acids or weak bases.

3. Toxins which are very firmly bound with the bacterial cells. For extraction, they are broken down by mechanical means, ultrasonic vibrations, alternate freezing and thowing, digestion by enzymes and chemicals. The second and third groups represent endotoxins which include the majority of bacterial toxins.

The majority of protein bacterial toxins catalyse certain chemical processes, break down vitally important compounds, are active in extremely small doses, have a latent period and inhibit the defensive functions of tissues. Some bacterial toxins have the properties of lecithinase. Thus, for example, *Clostridium perfringens* produces exotoxin (lecithinase C) which is able to cleave lecithin into phosphorylcholine and a diglyceride. Necrosis of the muscular tissue is caused as a result of the combined action of lecithinase, collagenase and mucinase (hyaluronidase). Collagenase and mucinase decompose the connective tissue of the muscles, and licithinase dissolves the lecithin of the membrane of muscle fibres. Haemolysis during gas gangrene takes place due to lysis of lecithin of the stroma in erythrocytes.

Bacterial toxins are characterized by organotropy (monotropy and polytropy) due to which the toxigenic microorganisms bring about tissue necrosis in localized foci of the causative agent. The necrotic manifestation of toxins has a great adaptive significance for the causative agent. Firstly, the toxins change live and reactive tissue into a substrate harmless for the pathogenic microbes. Secondly, the necrotic tissue protects the parasite from the effects of the defense reactions of the macroorganism.

Toxins are regarded as enzymatic poisons, which are capable of arresting metabolic processes. This view point is considered to be the most probable. It has been suggested that in the process of development of saprophytic bacteria entering into long symbiosis with animal organisms, the ability to produce enzymes, facilitating symbiosis with tissues, and increasing their life activities at the expense of the host, was gradually raised. Eventually, due to the establishment of the parasitic mode of life, the enzymes of these bacteria became more specialized as a result of which adaptive enzymes transformed into enzymatic toxins—exotoxins. Therefore, it can be considered, that toxic infections were formed in a later period, preceded by a simple parasitism and a disease of a septic type.

According to A. Pappenheimer, the diphtheria toxin is an incomplete product of the cytochrome B synthesis. Diphtheria toxin and the protein component of cytochrome B of diphtheria bacilli are related in origin and structure. If the nutrient medium contains 0.5 mg/l iron, the diphtheria bacteria excrete neither porphyrin nor toxin. If the concentration of iron in the nutrient medium is less than 0.1 mg/l, then the diphtheria bacteria begin to produce porphyrin and the protein component (toxin) of cytochrome B, but excrete them into the surrounding medium, as without iron the complex protein cannot be produced. It is possible that toxin production is associated with lysogenicity of the culture. This association is quite clearly expressed in diphtheria bacteria of the gravis variety.

However, the direct dependence between lysogenicity and toxigenicity was not established. Many strains of diphtheria bacilli may be lysogenic and nontoxigenic and vice versa, toxigenic cultures are not always lysogenic.

Exotoxins are capable of causing *potentiation* when, under the influence of a mixture of toxins, their action in the organism is more marked. An especially distinct potential action is shown by toxins of organisms responsible for gas gangrene and tetanus and of staphylococci, diphtheria bacilli and staphylococci.

Glucide-lipid toxins. These toxins are found in Gram-negative (enteric fever, paratyphoid and dysentery organisms, vibriones, brucellae, meningococci, etc.), but not Gram-positive bacteria.

According to their chemical composition, glucide-lipid toxins are polysaccharide compounds (50-65 per cent) with fatty acids (20-25 per cent) containing acetic and phosphoric acids.

During further investigation it became evident that these kinds of toxins are not pure glucide-lipid compounds, but contain nitrogen compounds and proteins, and they are thus related to the glucide-lipid-protein complexes.

Polysaccharide toxins. Many scientists have extracted toxic substances from bacteria which do not contain proteins and which, according to composition, are specific polysaccharides. They differ from usual polysaccharides in that they contain glucose, galactose, mannose, arabinose, rhamnose, aminosaccharides, lipids and other substances.

Later, it was revealed that probably there are no polysaccharide toxins completely free of proteins, however, there is strong evidence that some toxins (streptococcus, meningococcus and dysentery) contain a large amount of carbohydrates. Type specificity revealed in toxic and immune reactions is associated with polysaccharides.

Polysaccharide toxins, in spite of large variations in chemical composition, are characterized by haemolytic, leucotoxic and neurotropic properties.

Some protein toxins cause haemolysis of erythrocytes (pneumococci, staphylococci, streptococci, etc.). The pneumococcal haemolysin is freed from the cells during autolysis. Intravenous injection into guinea pigs is lethal. During subcutaneous injection in small doses it brings about the production of antibodies in the organism. The toxin is inactivated by cholesterol, pepsin, papain and trypsin. The haemolytic activity of the pneumococcus toxin is determined by the degree of haemolysis of a 1 per cent suspension of erythrocytes. According to physical and chemical properties, pure highly active haemolysin is grouped with the protein toxins. The mechanism of its action on erythrocytes is not clear. Some regard it as an enzyme and erythrocytes as a substrate, while others deny

the enzymatic nature of haemolysin. Streptococcal haemolysin has properties similar to those of pneumococcal haemolysin.

According to the mode of action on erythrocytes, there are alpha- and beta-haemolysins. Microbes producing *alpha-haemolysin* cause the production of green or dark-green colonies of microbes on blood agar, as a result of the haematometamorphosis of the iron in erythrocytes. *Beta-haemolysin* dissolves erythrocytes, and upon the cultivation of bacteria producing beta-haemolysin, transparent zones of haemolysis are formed around the colonies.

Besides, a number of pathogenic microbes produce gamma-haemolysin which causes the haemolysis of erythrocytes in rabbits, humans, guinea pigs and is characterized by poor resistance to heating. Also, delta-haemolysin has been discovered which destroys the erythrocytes of man and some animals. It is excreted, for example, by pathogenic strains of staphylococci.

Staphylococci and streptococci produce *leucocidins* which destroy pleomorphonuclear leucocytes.

Pathogenic strains of bacteria produce *coagulase* which causes coagulation of human, horse and rabbit plasma. Coagulase does not clot the plasma of guinea pigs, rats and chickens.

Some bacterial enzymes have toxic properties. Thus, for example, more than 200 species of microbes (bacteria of pneumonia, ozaena and rhinoscleroma, *Proteus*, continental strains of causative agents of plague, etc.) produce *urease*, which proved to be a toxic enzyme.

Many amino acid *decarboxylases* produced by causative agents of gas gangrene and other microbes have toxic properties.

Lecithinases subdivided into A, B and C are typical enzymatic toxins. *Lecithinase A* is found in snake, bee and scorpion venom, *lecithinase B*—in plants, and *lecithinase C*—in many pathogenic microbes, especially in some causative agents of gas gangrene.

Clostridium perfringens produces a typical alpha-toxin, which is considered to be a specific bacterial enzyme.

Some microbes produce toxic substances: methylamine, dimethylamine, histamine, choline, neurine, etc. Toxic amines are products of the decomposition of bacterial protein, and may accumulate in spoiled foodstuffs and serve as a source of food poisonings.

A number of microorganisms produce *ammonia* and cause ammonia intoxication (*Clostridium histolyticum*, etc.). Ammonia is produced by deamination of amino acids.

Toxins of rickettsiae and viruses. Rickettsial toxins are relatively labile substances, closely bound with the cells of the rickettsiae themselves. They comparatively quickly disintegrate after the death of rickettsiae from the action of formalin or heating at 56-60°C for 30 minutes.

Human pathogenic viruses also contain toxic components. They have been found in causative agents of influenza, parotitis, etc. Viral toxins are thermolabile, sensitive to the action of formalin

and other substances. Toxic substances of the influenza virus are revealed upon the injection of the virus into the anterior eye chamber of the rabbit or guinea pig. Within a day the iris swells and hyperaemia occurs, and injection of the blood vessels also takes place. In 2 days the cornea becomes opaque. Rickettsial and viral toxins are easily neutralized by specific immune sera.

Toxins cause distortions in metabolism, causing changes in the adrenalin and ascorbic acid levels. Under the influence of toxins, profound inhibition of such an important link in metabolism as the Krebs oxidation cycle of tricarboxylic acids occurs.

Local as well as general manifestations of intoxications are accompanied by morphological changes in the formed elements of the blood, in the composition of proteins, enzymes, in the serological (production of antibodies), general clinical (temperature and neuropsychic) reactions, in disturbances in the respiratory organs, cardiovascular system, etc. Anatomical changes are characterized by inflammatory processes in the lymph nodes or by affection of certain organs and tissues.

Invasive properties of pathogenic bacteria. Virulent microbes are characterized by the ability to penetrate tissues of the infected organism.

By chemical analysis it has been established that the greater part of the main substance of connective tissue contains polysaccharide — *hyaluronic acid* which is capable of resisting penetration into the tissue of different foreign substances, including pathogenic microbes.

This protective barrier of connective tissue can be overcome due to the disintegration of hyaluronic acid by toxic substances of animal, plant and microbial origin. In 1928 F. Duran-Reynals established that upon infection of a rabbit with the vaccinia virus (cowpox), the infectious process increased considerably, if together with the virus aqueous extracts of rabbit, guinea pig or mouse testes were injected intracutaneously. Later on, it was established that factors capable of increasing the permeability of tissues are found in some bacteria, snake venom and in different tissues and organs of animals. Substances causing this change in the permeability of tissues are known as *spreading factors*.

A certain enzyme was isolated from different tissues which hydrolysed hyaluronic acid. Some spreading factors are similar to this enzyme which is known as *hyaluronidase*.

Spreading factors are characterized by an extremely high activity. They act in very small doses, disintegrate at 60°C within 30 minutes, and have enzymatic properties.

Spreading factors are not confined to hyaluronidase. They include substances differing in nature. They include fibrinolysin produced by haemolytic streptococci of the A group, pathogenic staphylococci, and organisms involved in gas gangrene, etc. Spreading

factors increase the local primary action of pathogenic microbes affecting the connective tissue, and enhance the development of a general infection. They were found in many pathogenic microorganisms (staphylococci, streptococci, pneumococci, causative agents of gas gangrene, tetanus, diphtheria, etc.).

The effect of spreading factors on the course of infectious diseases is related to the virulence of the causative agent. In weakly virulent microbes such as staphylococci, colibacilli, and *Proteus*, the spreading factor increases the infectious process, if a large quantity of the microbes are injected. In highly virulent causative agents (*Mycobacterium tuberculosis*, type I pneumococcus) spreading factors increase the infectious disease with a minimal number of bacteria, sometimes with only several individuals.

The role of capsular material in bacterial virulence. Some pathogenic microorganisms (bacilli of anthrax, *Clostridium perfringens*, pneumococcus, causative agents of plague and tularaemia) are capable of producing a capsule in animal and human bodies. Certain microorganisms produce capsules in the organism as well as in nutrient media (causative agents of rhinoscleroma, ozaena, Friedländer's pneumonia).

Capsule production makes the microbes resistant to phagocytosis and antibodies, and increases their invasive properties. Thus, for example, capsular anthrax bacilli are not subject to phagocytosis, while noncapsular variants are easily phagocytized.

The high virulence of capsular microbes is associated with the toxic substances contained in the capsule.

In chemical composition, the capsular material in some microbes consists of complex polysaccharides, and in others it consists of proteins. It can be different in separate strains of the same species, and on the other hand may be similar in different bacteria. There are nitrogenous and nitrogen free compounds in the capsular polysaccharides. They give the microorganisms type specificity. In types II and III pneumococcus, the capsule is a glucoside of cellobiuronic acid in a highly polymerized state. In types I and IV the capsule contains highly polymerized compounds of aminosaccharides and organic substances. In some bacilli the capsule consists of polypeptides of d-glutamic acid, while in bacteria of Friedländer's pneumonia it is a polymer carbohydrate, and in anthrax bacilli it consists of glucoprotein.

Bacterial aggressins. Besides toxigenicity, invasiveness and capsule production, pathogenic microbes are capable of producing substances which inhibit the defense mechanisms of the organism, and increase the pathogenic action of many causative agents of infectious diseases. O. Bail named them aggressins. They were found in peritoneal and pleural exudates of laboratory animals infected with anthrax bacilli, pneumococci and other microbes. Aggressins alone, separated from bacteria and exudate cells by filtration,

upon injection into the animal are harmless, but upon their addition to a nonlethal dose of the microbes, a severe infectious process develops, often ending in death of the animal.

Aggressins were found in causative agents of enteric fever, paratyphoids, cholera, anthrax, diphtheria, plague, tuberculosis and pyogenic diseases.

The nature of aggressins has not been completely determined. E. Zauerbeck considers Bail's aggressins to be toxic substances in microbial cells. E. Rosenau calls them *virulins*. N. Chistovich and V. Gurevich obtained from different cultures substances (antiphagins) capable of inhibiting phagocytosis.

Antiphagins are characteristically thermostable, but decompose upon boiling within 20 minutes. They have been obtained from a suspension of agar cultures in an isotonic solution. N. Gamaleia discovered complexes in pleuritic exudate which increase the virulence of pathogenic microbes and named them predisposins. It is possible that the defense antigen of anthrax bacilli is an extracellular enzyme inhibiting the phagocytic reaction.

V. Brown and colleagues established that virulent bacteria produce a special substance in the host body which stimulates their growth and destroys the nonvirulent types of these bacteria. It is produced by bacteria from the breakdown products of desoxyribonucleic acid.

Mechanism of virus infections. Viruses are known to be intracellular obligate parasites. The mechanism of the interaction of the virus with the susceptible cell is a rather complex cycle. It consists of five phases: (1) adsorption of the virus onto the surface of sensitive cells; (2) penetration into the cell of the virus or its nucleic acid bearing the function of genetic information; (3) blocking of cell information; (4) synthesis of virus components; (5) release of viruses from the cell.

Adsorption of the virus (as has been proposed) takes place when it becomes bound to the cell receptors which are composed of mucopolysaccharides or lipoproteins. This process continues for 10-60 minutes. The viruses are not freed from the susceptible cells, while the insusceptible cells do not retain the virus firmly. Some scientists explain the insusceptibility to the virus as a lack of specific receptors without which adsorption does not take place.

There are several points of view concerning the mechanism of *the penetration of the virus* into the cells of tissues or organs. Some scientists consider that a fully developed virus consisting of nucleic acid and protein penetrates into the cell, although the protein does not take part in the further development of the infectious process. According to another point of view, only the nucleic acid which frees itself of the protein prior to incorporation penetrates into the cell. A number of scientists have established that ribonucleic acid of virulent and weakly virulent strains of viruses obtained

by deproteinization has genetic information to a similar degree. It freely penetrates the cell by passing the adsorption phase and carries out the synthesis of the viral nucleic acid and protein.

In the third and fourth phases in the development of the infectious process an inhibition of the genetic information in the cell and synthesis of viral components take place. Synthesis completed within a few hours is characterized by the production of nucleic acid in the nucleoles of the infected cell, and by its entrance into the cytoplasm. It is bound by the ribosomes in which nucleoprotein is produced which then unites with the lipids and polysaccharides of the cell. Multiplication of the virus takes place in the cytoplasm of the infected cell. Therefore, under the influence of the virus an excess accumulation of the protein and a disturbance in the coordination of cellular synthesis take place which leads to death of the cell. According to the data of other scientists the production of viral nucleic acid occurs in the nucleus, and the synthesis of protein in the cytoplasm. Some viruses (virus of herpes simplex, adenoviruses, etc.) are produced in the nucleus of the infected cell. The mature virus upon leaving the cell acquires a lipid-polysaccharide wall.

The excretion of the virus from the cell takes place in various ways. In some virus diseases the excretion of the virus is accompanied by the disintegration of the cell, while in others neither the nucleus nor cytoplasm is disintegrated. The cytopathogenic effect is characterized by a variety of morphological manifestations: the injury of the nucleus due to adenoviruses, injury of the cytoplasm during influenza, or injury of both the nucleus and cytoplasm during poliomyelitis and encephalitis.

Thus, in the mechanism of viral infections an interaction of two genetic systems (genomes)—viral and cellular—takes place. In some cases the virus causes death of the cell or injures it, in other cases a neoplastic process occurs. As has been noted already the interaction of the virus genome with the gene of the host cell commences after the penetration of the virus into the cell, and its deproteinization by nucleic acid. In most cases the functions of the cell nucleus are inhibited. In viruses containing DNA the genetic code is associated with DNA, and its expression, with RNA. In viruses containing RNA both functions are united in the same molecule of RNA, while the inhibition of the cellular genome is carried out by histones which are synthesized in the ribosomes soon after deproteinization of the viral RNA. These histones enter the cell nucleus and block the DNA averting the synthesis of cellular informational RNA, as a result of which the stream of information from the cell nucleus into the cytoplasm supplying the processes of metabolism ceases or is arrested. Its subsequent work is programmed by the viral genome. Thus, the viral RNA freed of its protein walls inhibits the work of the cellular genome, induces

the synthesis of its own polymerase and the reproduction of the viral RNA, and synthesis of structural proteins commence, which terminates in the formation of mature virus particles. These processes are performed in a definite sequence: synthesis of early proteins, replication of viral RNA, synthesis of late (structural) proteins.

However, the interaction of the virus and cell is not exhausted by the above mentioned mechanism. Thus, for example, upon injection of the smallpox virus by pinocytosis, the enzymes of the cell wall disintegrate only the outer walls, leaving the nucleotide of the virus untouched. The proteins of the outer walls or products of their disintegration diffuse into the cell nucleus, induce the work of the gene (cistron) responsible for the synthesis of the enzyme with the help of which the disintegration of the nucleotide proteins of the smallpox virus takes place. The nucleic acid is released and later carries out the formation of virus particles.

For the viruses of influenza and parainfluenza diseases, there is another type of interaction with the host cells in that the cell proteins are included in the composition of viral particles. The nucleocapsid of these viruses which has a spiral tubular structure is composed completely of specific viral components (nucleic acids and protein). Besides haemagglutinin, the outer walls of the virus are composed of proteins and lipids of host cells.

In tumours caused by viruses (the Rous sarcoma, Shope papilloma, etc.) a characteristic change is hyperplasia or an excessive tissue growth. Multiplication of viruses during the above mentioned diseases is accompanied by a stimulation in the growth of cells which leads to the development of tumours and necrosis of the cells of infected tissues.

In certain viral diseases it has been established that viruses have the capacity to cause intracellular changes with the production of a certain kind of inclusions. They are localized only in the cytoplasm (Guarnieri's inclusion bodies in vaccinia, and the Babes-Negri bodies in rabies, Bollinger bodies in fowl-pox, also in foot-and-mouth disease, and human warts) or only in the nucleus (in herpes, chickenpox, poliomyelitis, yellow fever, tick-borne spring-summer encephalitis, epidemic hepatitis, measles) or in the nucleus and cytoplasm (in smallpox). Inclusion bodies are quite varied in composition, viral particles being enclosed in most of them. Some inclusion bodies serve as a diagnostic character.

THE ROLE OF THE MACROORGANISM, ENVIRONMENT AND SOCIAL CONDITIONS IN THE ORIGIN AND DEVELOPMENT OF THE INFECTIOUS PROCESS

The origin of the infectious disease depends on the reactivity of the human body, the quality and quantity of the causative agent, and the influence of the external environment and social conditions.

The penetration of the causative agent into the body does not always entail disease but in many cases it is limited by a short-term infection without any manifestation of the disease or by a comparatively long carrying state (pneumococcus, adenoviruses, enteroviruses, herpes virus, malarial plasmodium, *Entamoeba histolytica*).

The reactivity of the human body with its immunobiological readiness to render the pathogenic microorganism harmless is closely related with the environment, with conditions of life, nature of work and nutrition, hygienic and general cultural level and many other factors.

The condition of the macroorganism and its resistance have a decisive significance in the origin, course and outcome of the infectious disease.

Susceptibility depends to a certain extent on *age* and *sex*, due to certain physiological peculiarities. For example, during menstruation, pregnancy and labour the female organism becomes more sensitive, particularly to streptococcal diseases.

Children are more susceptible to some infectious diseases, and less susceptible to others than adults. Resistance to many infectious diseases in children up to the age of 6 months is associated with a poorly developed central nervous system, and also with the presence of maternal immunity. Besides, it has been established that in relation to some diseases (dysentery, staphylococcal and streptococcal diseases, colienteritis and infections caused by Coxsackie virus) children are more susceptible than adults. The varied age resistance to infectious diseases depends on the nature of metabolism, the function of the organs of internal secretion, and on peculiarities of immunity.

Such factors as nature of nutrition (general starvation, deficiency of proteins, fats, carbohydrates, vitamins, and trace elements), overstrain, cooling, sanitary-hygienic conditions of work and life, also various somatic diseases, chronic poisonings and disturbances in the normal activity of the central nervous system have the effect of increasing the susceptibility to infectious diseases.

General starvation is accompanied by an aggravation of tuberculosis, dysentery, furunculosis and other diseases. As a result of starvation not only individual, but specific immunity is lost. For example, during starvation, pigeons become susceptible to anth-

rax to which they are resistant in a normal state. Lowering of the resistance in animals is not only due to general starvation, but also due to a deficiency of individual components of food, e.g., proteins, fats, carbohydrates. Starvation is accompanied by a disturbance in the protein metabolism, which leads to a decrease in the synthesis of immune globulins (antibodies), and a lowering of the activity of phagocytes.

Vitamin deficiencies have a great influence on the susceptibility to infectious diseases. A deficiency of vitamin A provides for the appearance of catarrhs of the mucous membranes of the eye and leads to xerophthalmia, enhances the development of skin affections, bronchopneumonia, influenza and acute catarrhs of the upper respiratory tract. A deficiency of vitamin B_{11} causes an increased susceptibility to leprosy and to a number of pathogenic and conditionally pathogenic microbes. Vitamin C deficiency causes a decline in the resistance to tuberculosis, diphtheria, streptococcus, staphylococcus, pneumococcus and other diseases.

Quite important is the fact that during many infectious diseases as a result of the lethal action of drugs on the normal intestinal microflora which supply the organism with vitamins of the B group, vitamin deficiencies develop.

In the past years great heed has been paid to the problems of the study of *mineral metabolism*. A deficiency of iron, calcium, magnesium, copper, zinc, iodine, manganese, boron, cobalt and molybdenum leads to a disturbance in metabolism, a decrease in the resistance of the organism and an increase in the susceptibility to infectious diseases. Small amounts of trace elements are capable of increasing the defense mechanisms of the macroorganism, in particular, the phagocytic activity of leucocytes. They restore the previously impaired biochemical functions.

Physical and mental overstrain associated with an irregular organization of working hours and a disturbance of conditions of life causes a weakening of the defense mechanisms to many infectious diseases.

Cooling lowers the resistance of the organism in relation to pathogenic and conditionally pathogenic microbes, enhances the development of pneumonia, catarrhs of the upper respiratory tract and other diseases. L. Pasteur proved that cooling in chickens causes a disturbance of specific immunity to anthrax. When the environmental temperature increases, penguins die from autoinfections caused by aspergilli.

Cooling as well as overheating of the body of animals leads to disturbances in biocatalytic reactions, a weakening of the organism and lowering of immunity to infectious diseases.

It is known, for example, that acute catarrhs are observed in the autumn-winter period, while colienteritis and infections caused by Coxsackie and ECHO viruses develop in the summer.

The action of ultraviolet rays and sunlight on the organism depends on the wave length, intensity and duration of application. Observations have shown that sunlight has a favourable effect on the organism, and to a certain degree increases the resistance to infectious diseases. However, in a number of cases, lengthy and intense irradiation is accompanied by a decrease in the resistance of the human organism to a number of pathogenic microbes. For example, spring relapses of malaria are observed in people infected by plasmodia and exposed to intense solar radiation.

Of great theoretical and practical importance is *the action of ionizing radiation.* As has been established small doses of X-rays increase the resistance of animals to various diseases, while increased doses lower it and enhance the activity of normal microflora and development of bacteraemia and septicaemia. At the same time the permeability of mucous membranes is disturbed, their barrier capacity is reduced, and the function of the reticuloendothelial system and defense properties of the blood are sharply lowered.

Especially dangerous to man are increasing doses of ionizing radiation as a result of the testing of nuclear weapons. Radioactive strontium accumulates in the atmosphere. It causes deep changes in the haemopoietic function of the bone marrow, the formation of tumours and impairs reproductive ability.

Poor *sanitary hygienic conditions* of work and life have an unfavourable effect on the human body. A deficiency of oxygen in the building, an excess of carbon dioxide and other harmful gases cause chronic poisoning and are favourable for the development of tuberculosis. The presence of dust in the air containing a large amount of silicates disturbs the integrity of the mucous membranes of the respiratory tract, increases the possibility of infection by different microorganisms, and leads to such diseases as tuberculosis, actinomycosis, aspergillosis, etc. Limited insolation also causes various disturbances in the activity of the body and enhances the development of diseases.

Besides these harmful external factors, a great influence on the susceptibility to infectious diseases is caused by various *somatic diseases* (diabetes and other disturbances of the organs of internal secretion, diseases of the cardiovascular system, liver, kidneys, chronic poisonings by alcohol, nicotine and other poisons).

The hypophysis-adrenal system is of great importance in maintaining stability of the internal medium of the organism. The system is stimulated by the action of different stimulants, e.g., mechanical traumas, cold, heat, ultraviolet and ionizing radiation, microorganisms, etc. As a result of an excess, deficiency or abnormal combination of hormones such as STH (somatotrophic hormone), ACTH (adrenocorticotrophic hormone), various disturbances in the functions of the organism may occur (see p. 223). Thus, for example,

cortisone inhibits the inflammatory reaction, and therefore enhances the development of the infectious process. The somatotrophic hormone, on the other hand, activates the inflammatory process and causes an anti-infectious action.

Disturbances of the normal activity of the central nervous system deserve special attention. As is known, the causative agents of infectious diseases are extraordinary biological stimulants.

With experimental infections, principally by neurotropic stimulants, it had been observed long ago that the injection of the infected material into the brain is accompanied by the greatest number of deaths.

Mental disturbances also lower the regulating function of the central nervous system. The mental patients in psychiatric hospitals more often contract infectious diseases.

Under the influence of various *national disasters* (hunger, war, earthquakes, floods) infectious diseases attain a mass distribution and are accompanied by a high death-rate and disability.

Thus, the infectious process reveals itself in the unity of biological and social factors. The disease incidence, severity of the clinical course and death-rate depend closely on the activity of the main economic laws of social formations.

MECHANISMS OF THE TRANSMISSION OF CAUSATIVE AGENTS, AND CLASSIFICATION OF INFECTIOUS DISEASES

In the process of evolution pathogenic microbes have evolved the ability to penetrate the human body in several ways, and to localize selectively in the tissues and organs in which they develop, causing a specific response reaction of the macroorganism.

In medical microbiology the aetiological principle based on the specific action of pathogenic microorganisms serves as the basis for the classification of infectious diseases. Since the amount of pathogenic species of microbes is comparatively great, it has become necessary to group all infectious diseases according to a certain principle, that is, according to the mechanism of transmission from the source of infection to the susceptible human body. In the Soviet Union the classification proposed by L. Gromashevsky has been accepted (see Table 10). I. Elkin and V. Zhdanov and co-workers have supplemented it by subdividing each group of nosological units into two series: *anthroponoses*, diseases peculiar to man, and *zoonoses*, diseases inherent in animals, but to which man is susceptible.

Thus, for each causative agent, there are definite mechanisms of transmission providing for its localization. Not only are the routes of penetration of pathogenic microorganisms specific, but

Table 10

The Length of the Incubation Period of the Main Groups of Infectious Diseases

Name of disease	Length of the incubation period in days			Notes
	average	minimum	maximum	
Intestinal infections				
A. Anthroponoses				
Enteric fever	14	7	21-28	
Paratyphoid A	8	2	14	
Paratyphoid B	6	3	15	
Dysentery	3	2	7	
Amoebiasis	20-30	2	45	
Cholera	2-3	Several hours	6	
B. Zoonoses				
Food poisoning (salmonellal)	6-24 hours	2-3 hours	1-2	
Botulism	1-2 days	2-3 hours	10	
Brucellosis	14	7	14-30	
Leptospiral jaundice	7	3-4	20	
Infections of the respiratory tract				
Influenza	2	Several hours	3	
Scarlet fever	5-7	1	12	
Diphtheria	5	2	10	
Measles	10-11	6	18	In those inoculated with antimeasles gamma-globulin—28 days
Whooping cough	9	2	15	
Chickenpox	14	10	21	
Smallpox	13-14	5	17	
Epidemic encephalitis	21	14	28	
Epidemic parotitis	18	3	30	
Epidemic meningitis	2-3	Several hours	7	
Leprosy	3-5 years	1 year	8 years	Up to 25-35 years
Blood infections				
A. Anthroponoses				
Epidemic typhus fever	10-14	5	21	
Relapsing fever	5-7	2	14	

Name of disease	Length of the incubation period in days			Notes
	average	minimum	maximum	
Malaria	12	6	31	During tertian malaria sometimes up to 11 months
Dengue fever	5-9	4	15	
Yellow fever	4	1	12	
Mosquito fever	5	2	8	
B. Zoonoses				
Q fever	19-20	14	26	
Relapsing fever (tick)	7	5	10	
Tick-borne encephalitis (spring-summer)	14	8	23	
Japanese encephalitis (autumn)	14	4-7	21	
Trypanosomiasis	12	8	21	
Infections of the skin integuments				
A. Anthroponoses				
Gonorrhoea	3	1	14-21	
Syphilis	21	10	50	
B. Zoonoses				
Tetanus	7-10	1	40	Sometimes up to a year and more (during operations of removing splinters)
Gas gangrene	3	1	—	
Glanders	7	3	14	
Melioidosis	2-3	2	14	
Foot-and-mouth disease	4	2	6	
Rabies	40	12	80	Up to one year and more
Trachoma	8	5	14	
Infections with different mechanisms of transmission				
A. Anthroponoses				
Poliomyelitis	7	3	10	
Epidemic hepatitis	21-26	2-14	50 (150)	
Infections caused by Coxsackie organisms	2-5			
Colienteritis	3-6	1	22	

Name of disease	Length of the incubation period in days			Notes
	average	minimum	maximum	
B. Zoonoses				
Plague	3-4	Several hours	9-10	In those vaccinated and after injection of the serum or streptomycin—up to 12 days
Anthrax	1-3	Several hours	8	
Tularaemia	3-8	1	21	
Leishmaniasis	21	10	9 months	
Ornithosis	10	7	15-25	

also the mechanisms of excreting them from the body. These peculiarities of causative agents of infectious diseases are taken into account when carrying out antiepidemic and preventive measures.

Course of the infectious disease. The dynamics of the development of the infectious process consists of the incubation and prodromal periods, the height of the disease and period of recovery (convalescence). A certain period of time elapses from the moment of penetration of the pathogenic microbe to the onset of the first signs of the disease, which has been named the incubation period of the disease. It varies from several hours (in cholera, toxinfections and plague) to several months and years (in leishmaniasis, leprosy).

The duration of the incubation period depends on the degree of the general and specific immunity of the human body, its reactivity, sensibilization (increased sensitivity), influence of harmful environmental factors and social conditions of life, and on the dose and virulence of the causative agent.

Reproduction and accumulation of the microbes and their toxins, the sum of the resulting irritations, the increase of the reactivity of the human body to the causative agent and its toxins take place during the incubation period. Infection can end with the development of the disease. The disease will not develop if the organism proves to be capable of actively mobilizing its defense forces, and of rendering harmless the introduced causative agent.

Infections with different mechanisms of transmission include tuberculosis, bartonellosis, toxoplasmosis, etc.

In some diseases *the prodromal period* ensues after the incubation period during which characteristic symptoms of the disease are absent and nonspecific characters develop common to many diseases (indisposition, loss of appetite, weakness, sometimes subfeb-

rile temperature), with the exception of measles (red spots on the mucous membrane of the mouth, Filatov's spots), and smallpox (prodromal rash on the face and limbs).

At the height of the disease, when the infectious process attained its highest intensity, it remains at such a level for some time the duration of which depends on the type of disease. During a favourable course, the disease enters the stage of convalescence, while in some cases the disease terminates in *a crisis*, involving a rapid decrease in temperature accompanied by sweating and frequently by the phenomenon of vascular collapse. In other cases convalescence is characterized by *lysis*, a gradual lowering of the temperature and lessening of the disease.

The most typical features of the infectious disease are fever, inflammation and affection of the central and vegetative nervous systems. Besides, there are functional and organic disturbances in the respiratory, digestive and urinary organs, and in some infections, dermal changes such as various rashes.

According to V. Menkin during inflammation the injured cells excrete: (1) leucotaxin, increasing the permeability of the capillaries, and thus enhancing a subsequent migration of leucocytes into the inflamed zone; (2) a factor causing leucocytosis during inflammation—a thermolabile alpha-globulin, acting directly on the bone marrow, and causing the entrance of immature granulocytes into the blood; (3) necrosin, a factor causing the necrosis of tissues; (4) pyrexin, a polypeptide causing a feverish reaction during inflammation; (5) leucopenin, a factor decreasing the number of leucocytes during inflammation; (6) exudin, a polypeptide stimulating the exudation in the later stages of inflammation.

The above mentioned reactions do not include all the mechanisms of inflammation in which a great role is played by phagocytosis, a reaction of the regionary lymph nodes, antibodies and other factors.

The inflammatory reaction is regulated by the hormone system (hypophysis-adrenal system). Upon the liberation of the adrenocorticotrophic hormone (ACTH) and corticoids (hormones of the adrenals), the inflammatory processes are inhibited. The production of the X-factor by the hypophysis and the excretion of anti-inflammation corticoids of the desoxycorticosterone type cause an increase in the inflammation potential of the whole body.

ROUTES OF TRANSMISSION OF MICROBES IN THE BODY

During the development of the infectious process microbes from the primary focus can enter the blood stream, and can be carried through the whole body. This condition is known as *bacteraemia*,

and during viral diseases—as *virusaemia*. Bacteraemia occurs with enteric fever, paratyphoid, brucellosis and other diseases.

In a number of infectious diseases *sepsis* or *septicaemia* (Gr. *septicos*—decaying, *haima*—blood) may occur with the infestation of many organs and tissues of the body by microbes (anthrax, plague, pyogenic and other septic diseases). Sepsis is characterized not only by the presence of pathogenic microbes and bacterial toxins in the organs and tissues, but by reactive phenomena accompanied by the inflammation and degeneration of cells. A characteristic feature of sepsis is that it occurs in the same clinical picture independent of the species of causative agent, which makes it difficult to establish a diagnosis of the disease according to its clinical course. The septic process accompanied by the production of purulent foci in different organs and tissues is known as *septicopyaemia*.

Some pathogenic (toxigenic) microbes which have become embedded in the skin integuments, mucous membranes, tissues and organs act on the organism predominantly with their exotoxins (causative agents of tetanus, botulism, diphtheria). This is known as *a toxaemia*.

Bacteraemia, septicaemia, septicopyaemia and toxaemia are sometimes accompanied by different changes in the tissues, in particular in the skin (rashes of different morphology).

Pathogenesis of the infectious process (mechanism of the origin and development of the disease) depends not only on the mass of bacterial cells and toxins, but also on the sum total of irritations caused by them.

Numerous investigations have proved that repeated introduction of small (subliminal) doses of a virulent culture or toxin causes a typical disease in animals, and in some cases death. If, for example, 0.01 Dlm of diphtheria toxin is injected daily into a guinea pig for 5-6 days, then it will perish, while a single injection of a total dose of toxin (0.5-0.6 Dlm) does not cause death. It has been revealed that the action of such small doses of toxin provides for *the summation of the toxic irritation* which depends on the dose of toxin (the force of irritation) and on the interval between injections (frequency of irritation). This picture is observed during intranasal infection of white mice which become ill and perish from the injection of $^1/_{100}$ or $^1/_{1,000}$ Dlm of the influenza virus within 5-6 days.

During hibernation of rodents (gophers, tarbagans, susliks, hamsters, bats, etc.) all the life processes are slowed down, physiological depression ensues and areactivity of all tissues and organs occurs. This provides for the resistance of animals to infection with pathogenic causative agents and tolerance to the action of different toxins.

FORMS OF MANIFESTATION OF INFECTIONS

According to manifestations, infections are subdivided into acute and chronic, obvious and latent, mixed and secondary.

Acute infections are characterized by a sudden onset and a comparatively short course (influenza, measles, scarlet fever, typhus fever and relapsing fever). Diseases with *chronic* or *protracted* courses include malaria, tuberculosis, syphilis, leprosy, brucellosis, amoebiasis, toxoplasmosis, and sometimes dysentery, etc.

Some infectious diseases can occur atypically, and latently without typical clinical manifestations. These forms of infection are called *latent* or silent during which the causative agent for a long period of time can be found in tissues or organs not causing clinically marked response reactions of the macroorganism. Most frequently, tuberculosis occurs in the latent form. Infection with the tubercle bacilli is many times greater than the disease incidence itself. During unfavourable effects of the factors related to the external or social environment, the latent form changes into the obvious typical form. In some cases, herpes, malaria, meningitis, poliomyelitis, etc., may occur latently.

The *asymptomatic* form of infection was named *inapparent* by C. Nicolle, in which clinical signs are absent, although at the same time multiplication of the causative agent takes place. Inapparent infection is an acute disease which terminates in convalescence after a certain period or in the disappearance of the causative agent from the body.

One of the forms of interrelationship which occurs between the pathogenic microorganism and a human or animal body without manifesting an obvious disease is *carrier state*. The ability of the causative agent to carry infectious diseases has been confirmed only in a relatively immune organism. Regarding specificity of action, carrier state has much in common with the infectious process. In some infectious diseases an intense and prolonged postinfectious immunity is produced which excludes carrier state (measles, smallpox, chickenpox, etc.). In other diseases during the period of convalescence a carrier state may be prominent which is different in frequency and duration (cholera, enteric fever, paratyphoid, dysentery, amoebiasis, scarlet fever, diphtheria, meningitis, malaria, encephalitis, poliomyelitis, etc.).

Carrier state may be found in healthy persons who have come into contact with diphtheria, meningitis, enteric fever, cholera, amoebiasis, encephalitis and poliomyelitis patients. Carrier state with a duration of 3 months is considered *acute*, while carrier state for longer periods is considered *chronic*. Prolonged carrier state (years and decades) has been described in enteric fever.

When infection occurs not with one species of causative agent, but with two or more, one speaks of *mixed infection* (measles and

scarlet fever, measles and tuberculosis). If the infectious process is caused by microorganisms changed under the influence of one or several co-members of the parasite coenosis, than this state is known as *parainfection.*

In some cases infection causes a weakening of the body which then becomes susceptible to other diseases. Thus, for example, after influenza or measles pneumonia occurs. This is known as *secondary infection.*

There are also focal and generalized infections. For example, during infection with staphylococcus, the infectious process causes furunculosis, and if the causative agent penetrates into the blood sepsis will develop. An alternate occurrence of focal and generalized infections is observed during tuberculosis and syphilis.

Reinfection is a repeated infection by the same species of microbe responsible for the disease which terminated in convalescence (gonorrhoea, syphilis, etc.).

Superinfection is a fresh infection of the body in which the main disease has not ended. Superinfection occurs in many infectious diseases in their acute and chronic forms.

Relapse is a return of the symptoms of the same disease (relapsing fever, paratyphoid fevers, etc.). Of certain significance in the occurrences of relapses is the low level of immunological activity of the organism during illness and convalescence.

Hereditary infectious diseases. Most scientists deny the possibility of hereditary transmission of infectious diseases in man by infected germ cells. However, the possibility of transmitting infectious diseases from the sick mother to the foetus through the placenta (staphylococcus diseases, syphilis, enteric fever and relapsing fever, toxoplasmosis, epidemic hepatitis, etc.) and during birth (blennorrhoea of the newly born) has been precisely established. Infections caused by latent viruses can be transmitted from the parents to the offspring.

* * *

In nature infectious diseases are subdivided into *exogenous* and *endogenous*. During exogenous infections, the causative agent penetrates the macroorganism from the environment (from patients, carriers, from foodstuffs, water, objects, air, soil, etc., which have been contaminated by them). Endogenous diseases (*autoinfections*) originate as a result of the activation of the indigenous microbes of the body (microflora of the skin, mucous membranes, respiratory and digestive tract, urogenital tract, eye conjunctiva) due to disturbances of the internal medium of the macroorganism as a result of external factors and social conditions.

The state of *autoinfection* is quite a widespread phenomenon. With a decrease in temperature the microflora of the upper respiratory tract is activated, which causes a different kind of inflammatory processes. Quite often the development of herpes is observed, the virus of which under normal conditions is inactive in the body, but during cooling, menstruation, disturbances in diet it becomes active and causes an infectious process. A certain role is played by radiation sickness in activating normal microflora, and is accompanied by bacteraemia.

Radiation lowers the bactericidal properties of the blood resistance and the production of antibodies, inhibits the phagocytic activity and inflammatory reaction and aids in sensitizing the body with products of tissue decomposition. Autoinfections include nasopharyngis, tonsillitis, appendicitis, conjunctivitis, pyogenic infections of the skin, otitis, cholecystitis, osteomyelitis, etc.

Under the influence of inflammatory-necrotic processes a C-reactive protein appears during the acute period in the patient's serum. It is produced as a result of tissue decomposition during inflammation, necrosis, and in particular during myocardial infarction and tumours. The discovery of this protein permits the differentiation of a number of acute inflammatory diseases and necrotic processes. Primary infection in such diseases as tuberculosis and brucellosis is exogenic, but the causative agent may remain in the body for a long time and not cause a disease. With the advent of unfavourable conditions for the macroorganism, the latent form of the disease becomes typical and causes relapses.

The intensity of the spread of infectious diseases. According to the extent of spread infectious diseases may be sporadic (separate diseases observed in a given area during a certain length of time).

A considerable increase in the level of sporadic incidence of a given disease is known as *an epidemic* (or epizootic in animals). When the epidemic reaches an unusually large size in some country or spreads over many countries or even continents, it is called *a pandemic*. In the VI and XIV centuries pandemics of plague were observed. From 1817 to 1925 there were six pandemics of cholera. In 1918-1919 a terrible pandemic of influenza (Spanish influenza) spread throughout all the countries of the world. In 1957 there was also a pandemic of influenza.

Besides, a special form of spread of infectious diseases exists known as *an endemic*, in which infectious diseases are retained for a long time in some locality (yellow fever, tick- and mosquito-borne encephalites, tick-borne rickettsioses, haemorrhagic fevers, leishmaniases, mosquito fever, tularaemia, amoebiasis, etc.). In contrast to endemic diseases there are exotic infectious diseases which are introduced from other countries (smallpox, cholera, etc.).

The morbidity rate of an infectious disease is estimated as the amount of infected per 10,000 or 100,000 of the population during the year. *The mortality rate* is determined as the total number of deaths from the given disease per 100,000 population. *Fatality* is expressed as a percentage of the number of dead per 100 infected.

Due to the success in the control of infectious diseases the total mortality rate in Europe has decreased in the last 100-150 years by 2-2.5 times, and in the Soviet Union by more than 4 times. The average expectation of life in 28 countries of the world is within the range of 70-75 years. In the last 50 years it has increased in the USA by 1.5 times, and in the USSR by 2.2 times.

Incidence of infectious diseases in the last 60-65 years has sharply declined. Infectious diseases as the cause of death come fifth after cardiovascular disorders, cancer and other malignant tumours, vascular disturbances of the central nervous system, and diseases of the respiratory tract.

THE STUDY OF IMMUNITY

The term *immunity* (Lat. *immunis*—freed from homage, save from something) usually means resistance of the body to pathogenic microbes, their toxins or to other kinds of foreign substances.

Immunity is a complex of physiological defense reactions which determine the relative constancy of the internal medium of the macroorganism, hinder the development of the infectious process or intoxication, and are capable of restoring the impaired functions of the organism.

Insusceptibility to infectious diseases depends on many factors grouped under the names of resistance and immunity. Resistance is the insusceptibility of the body to the effect of pathogenic factors. Resistance embraces a wider group of phenomena of insusceptibility than immunity. Nonspecific resistance is the insusceptibility of the body to injury by pathogenic factors: mechanical (traumas, rocking), physical (barometric pressure, cooling, overheating, radiating energy, ionizing radiation), chemical (oxygen deficiency, excess of carbon dioxide, action of poisonous substances, drugs, poisons of a chemical and bacterial origin), and biological (pathogenic protozoa, fungi, bacteria, rickettsiae and viruses).

There may be resistance of the entire body and of its separate systems, although mutual dependence of both exists. Resistance is associated with the anatomical-physiological characteristics of the body, development of the central nervous system, and endocrine glands. It depends on the phylogenetic development of the animal, the individual and functional state of the body, and in man it depends also on social factors. Mental traumas predispose to somatic and infectious diseases; chronic hunger and vitamin deficiencies lead to a decline in resistance; intoxication by alcohol, opium, cocaine and other narcotics have a negative effect on human resistance.

Resistance may pertain to the phenomenon of nonspecific immunity. In this case it considerably concedes to resistance in specific

immunity to infectious diseases produced by administration of biological preparations (vaccines and sera).

Numerous investigations have established that humans who had had an infectious disease were not infected when looking after patients with the same disease.

In the traditions and life of ancient people considerable allowance has been made for preventive measures including vaccines against various diseases. Thus, for example, the inhabitants of East Africa from time immemorial have successfully used vaccinations against the bites of poisonous snakes. For vaccines they used snake venom, contained in a paste from plants. The paste, applied to cross-like scarifications on the skin of the person being vaccinated, caused a prolonged inflammation, and after being absorbed gradually helped in producing immunity to the lethal bites of poisonous snakes. Repeated vaccinations were made over a period of several years. Africans produced an artificial immunity to tick-borne relapsing fever by natural immunization. They carried on their body ticks which had contained a virus for a long time.

The people of Mauritania (Western Africa) protected their herds from epizootic peripneumonia of cattle by vaccinations (skin cuts) with the aid of a dagger that had been dipped in the lung of a bull which died from peripneumonia. This method became known in Europe in 1773, and was widely employed by cattle farmers in England and Holland.

In South-East Asia, 2,000-3,000 B.C., children were vaccinated against smallpox. Iranians inoculated into skin cuts smallpox scabs which had been dried and ground into a powder. Cherkesses and Georgians made intradermal injections with needles, moistened with smallpox infectious material.

In remote ages for combating smallpox among many peoples, it was the custom to make children, who had scratches on their hands, milk cows infected with cowpox. This method was also known long ago in England, France and Germany.

E. Jenner learned of these folk methods of vaccinations against smallpox from peasants. For 25 years he checked this observation, and in 1798 published his discovery. However, only within 100 years after E. Jenner's work, *immunology* was enriched with its own methods built on the scientific works of L. Pasteur.

L. Pasteur and his pupils discovered a method of weakening the causative agents of chicken cholera, anthrax and rabies, and confirmed the possibility of using them for specific immunization. Attenuated microbes received the name of vaccines, while the method itself became known as vaccination (immunization).

During the last 80 years the problems of immunity have acquired not only a medical, but also a general biological significance. Immunological methods are used to determine infectious diseases, in forensic medicine, sanitary-hygienic practice, in the production of highly effective preparations used in therapy and prophylaxis of infectious diseases, and in establishing evolution and genetic links among plant and animal species.

As a result of numerous investigations in the field of immunity, the main principles of acquired immunity were formulated in accordance with the works of L. Pasteur, E. Metchnikoff and many other scientists.

E. Metchnikoff founded the biological theory of immunity as a system of defense reactions, formed in the process of evolution of

the animal world, in the struggle for existence and natural selection.

The present period in the development of immunology is characterized by the concept that the mechanisms of immunity are considered as a sum of all physiological reactions of the macroorganism, directly bound with the penetration of microbes and the products of their life activity.

TYPES AND FORMS OF IMMUNITY

Modern classification subdivides immunity into two types according to origin: (1) *species* (*heritable*) and (2) *acquired.*

Species immunity is insusceptibility of certain species of animals to diseases which attack other species. It is transmitted by heredity from one generation to the next. An example of species immunity is insusceptibility јo man to cattle plague, chicken cholera and infectious horse anaemia. On the other hand, animals are not infected by many human infections such as enteric fever, scarlet fever, syphilis, measles, etc.

Species immunity is the result of a long evolution of interrelations between the macroorganism and pathogenic microorganism. It depends on those biological peculiarities of a given species of organism, which were formed during historical development in the course of natural selection, variation and adaptation to the environmental conditions.

Acquired immunity is subdivided into *natural* and *artificial.* Natural immunity in turn is divided into (1) active that acquired following an obvious (postinfection) or latent disease or repeated infection without clinical manifestations; (2) passive immunity of newborn (maternal, placental), i.e., immunity in newly born children, acquired from the mother in the period of intrauterine development, through the placenta in the process of ontogenesis. The duration of immunity of the newborn is short. After about 6 months this immune state disappears and children become susceptible to many infections (measles, diphtheria, scarlet fever, etc.). Artificial immunity is reproduced by active or passive immunization (see "Humoral Immunity").

There are the following forms of immunity: tissue, humoral and functional immunity.

TISSUE IMMUNITY

This form of immunity which includes defensive properties is associated with phagocytosis, barrier function of the skin, mucous membranes, lymph nodes and other tissues and organs.

Phagocytosis. The most ancient form of immunity is phagocytosis, a defense adaptation which entails the seizure and digestion of foreign particles, including bacteria and remains of disintegrated cells, by *phagocytes*. The phenomenon of phagocytosis is of great importance in defense reactions of heritable and acquired immunity. E. Metchnikoff established that amoeboid cells of the mesoderm in transparent marine animals are capable of swallowing and digesting various foreign particles.

During early embryonic development amoeboid (mesenchymal) cells are produced between the epithelial cells, which do not take part in building up organs, from which all types of motile erythrocyte cells and various species of migrating leucocytes originate. They are contained in the thymus, bone marrow, spleen, lymph nodes, tonsils, appendix, and interstitial tissues of parenchymatous organs.

For more than a quarter of a century, E. Metchnikoff and his pupils accumulated facts confirming the defense role of phagocytosis during infection of vertebrate animals with pathogenic microbes. This provided for the possibility of establishing in the evolution and phylogenesis of cells the relation between digestion and phagocytosis. E. Metchnikoff subdivided those cells able to carry out phagocytosis into *microphages* and *macrophages*.

Microphages include granular leucocytes, neutrophils, eosinophils and basophils, of which only neutrophils have quite a marked ability for phagocytosis. Eosinophils and basophils are characterized by a weak phagocytic activity, although this problem has not yet been studied sufficiently.

Phagocytosis takes place with the help of macrophages which may be motile (monocytes of the blood, cells of the lymph nodes and spleen, polyblasts, histiocytes, etc.) or nonmotile (reticular cells of the spleen, cells of the lymphatic tissue, endothelium of the blood vessels, etc.).

At present it has been proved that many cells of the body are capable of phagocytic activity and these have been grouped by W. Taliaferro into a lymphoid-macrophage system.

Cells of the Connective Tissue, Taking Part in Immunological Reactions

1. Fixed cells

A. Fibroblasts and endothelial cells B. Macrophages 1. Reticular cells of reticular organs 2. Parietal cells of the sinuses of reticular organs and sinusoids of the liver, kidneys and hypophysis 3. Adventitial cells 4. Histiocytes of connective tissue, interstitial connective tissue of various organs—macrophages of the skin, cells of the stroma of the intestine and lungs, phagocytes of the brain glia	Macrophage system

2. Free cells	
A. Macrophages of inflamed tissues B. Intermediate polyblasts (transitional forms between nongranular leucocytes and macrophages of inflamed tissues) C. Nongranular leucocytes 1. Monocytes 2. Lymphocytes 3. Plasmatic cells	Macrophage system
D. Granular leucocytes 1. Heterophilic cells (neutrophils, pseudoeosinophils) 2. Eosinophils 3. Basophils	Microphages

The process of phagocytosis consists of four phases.

The first phase involves the approach of the phagocyte to the microbe by means of a positive chemotaxis. Under the influence of the products of the life activities of microbes excitation of the phagocytes occurs, which leads to a change in the surface tension of the cytoplasm, and gives the phagocytes amoeboid motility.

In the second phase adsorption of the microorganism on the surface of the phagocyte takes place. This process is completed under the influence of an electrolyte which alters the electrical potential of the phagocytized object (microbe).

The third phase is characterized by submergence of the microbe into the cytoplasm of the phagocyte, which seizes minute objects quite rapidly and large ones (some protozoa, actinomycetes, etc.) are engulfed in pieces.

In the fourth phase intracellular digestion of the engulfed microbes by the phagocytes takes place.

In the process of phagocytosis various changes in the microbes can be observed, e.g., the production of granules in cholera vibrios, swelling of enteric fever bacteria, fragmentation of diphtheria bacilli, destruction of anthrax bacilli and swelling of cocci. Eventually, the phagocytized microbes become completely disintegrated.

Factors which speed up phagocytosis include calcium and magnesium salts, the presence of electrolytes, and antibodies (opsonins and bacteriotropins). Phagocytosis proceeds more vigorously in the immune than in the nonimmune organism.

Toxins of bacteria, leucocidin, capsular material of bacteria, cholesterol, quinine, alkaloids and also a blockade of the reticuloendothelial system inhibit phagocytosis.

Besides *complete phagocytosis* (Fig. 57) *incomplete phagocytosis* is observed in certain diseases (gonorrhoea, leishmaniasis, tuberculosis, leprosy) in which microorganisms are absorbed by phagocytes, but do not perish, are not digested, and in some cases reproduce (Fig. 58).

The skin, mucous membranes and lymph nodes. The data published by E. Metchnikoff on phagocytosis created wide interest and called for the necessity of carrying out numerous experimental investigations, as a result of which the defense mechanism of the skin, mucous membranes, lymph nodes and cells of many tissues and organs was established.

In a normal, uninjured state, the skin not only is a true mechanical protective barrier, but a bactericidal factor. It has been established that the clean skin of a healthy person has a lethal action on

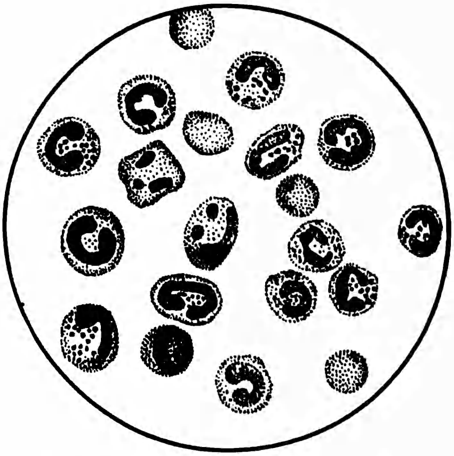


Fig. 57. Phagocytosis of staphylococci

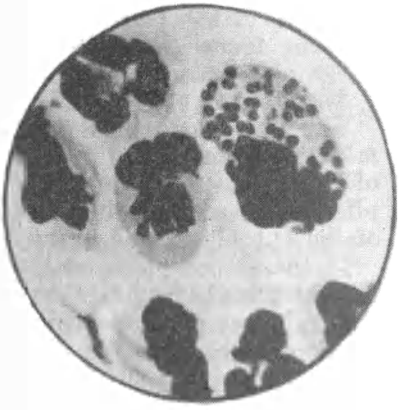


Fig. 58. Phagocytized gonococci

a number of microbes (haemolytic streptococcus, salmonellae of enteric fever and paratyphoid fever, colibacillus, etc.). Investigations confirmed that washing the hands not only aids in mechanically removing microbes from the surface of the skin, but also in increasing its bactericidal properties.

The mucous membranes of the eyes, nose, mouth, stomach and other organs have defense adaptations. Like the skin barrier, the mucous membranes perform antimicrobial functions as a result of their impermeability to different microbes and the bactericidal action of their secretions. In lacrimal fluid, sputum, saliva, blood, milk, tissues and organs an antibiotic, *lysozyme*, is found which is an enzyme similar to ribonuclease. It is found in some bacterial cells.

Due to the establishment of this defense mechanism the biological role of lacrimal fluid, saliva, nasal mucus and sputum becomes apparent. A lack of lysozyme in the tears affects the cornea. When animals lick their wounds they transfer lysozyme into them. Microbes which have penetrated into the mucous membranes are continuously destroyed by the action of lysozyme. Nasal mucus is bacte-

ricidal for many microbes and viruses of influenza, herpes, poliomyelitis, etc.

Bactericidal properties are not limited to the action of lysozyme. There are other antibiotics produced by the organs and tissues, which are capable of inhibiting microbes. A special substance *inhibin* has been found in the saliva, and the antibiotic *erythrin*—in the erythrocytes. Both preparations have a bacteriostatic action on diphtheria bacilli.

A substance of a protein nature, *interferon*, which has a lethal action on viruses is produced by the cells of some tissues (see p 227).

Of a certain significance in physiological immunity is *hyaluronic acid* which inhibits the penetration of microbes into tissues and organs. Gastric juice has quite marked bactericidal properties in relation to many causative agents, especially those of the *Salmonella* group and organisms responsible for food poisonings.

Besides the defense adaptations of the skin and mucous membranes a large role is played in natural immunity by *the lymph nodes* in which the pathogenic microbes penetrating through the injured skin and mucous membranes are localized and rendered harmless. *Inflammation* develops in the lymph nodes.

The inflammatory reaction is characterized by the liberation from the tissues of a number of substances (leucotaxin, leucopenic factor, histamine, serotonin, etc.) under the influence of which changes in the leucocytes occur. As a result they become sticky and adhere to the capillary wall, where they enter into the tissues. They enhance (induce) proliferation of adjacent cells. Leucocytes accumulated in the inflammatory zone produce a protective barrier which hinders the spreading of microbes into the tissues, blood and organs .Phagocytosis plays a great role in the blocking and destruction of microorganisms in the inflammation focus.

V. Menkin considers that a protein substance is secreted from the infected cells, which provides for the mobilization of immature leucocytes from the bone marrow and hyperplasia of its elements. Inflammation as a defense reaction is accompanied by a destruction of the causative agent, and the process ends in restoration of the infected lymph node.

It has been proved that the smooth muscles of the intestine and uterus have defensive properties against pathogenic microbes and their toxins. The mechanism of this defense consists in the decrease of reactivity in smooth muscles (the lowering of their sensitivity to the action of stimulants) under the influence of the infectious process or vaccination. This immunity is strictly specific and takes an active part in the general complex of defense reactions of the body. Besides lowered reactivity, smooth muscles may carry out a defensive function, as a result of increased sensitivity.

As has been established by G. Selier, the state of tissue reactivity regulated by adaptive hormones frequently determines whether the

organism will succumb to the disease or whether it will be resistant to the potential pathogenic agent. Not only pathogenic, but saprophytic microorganisms are capable of causing death in the animal, if the inflammatory reaction is inhibited by the action of anti-inflammatory corticoids (cortisone).

A number of investigators have established *the localizing capacity* of tissues, which can manifest itself without the participation of phagocytes and antibodies.

At present there are data confirming the ability of all tissue cells to defend themselves from pathogenic microorganisms and their poisons.

Tissue immunity related to nonspecific defense reactions is not an adaptation distinct from other systems of the organism. It is closely tied with all the defensive manifestations of the macroorganism, is subordinated to general physiological laws, and is controlled by the nervous system.

HUMORAL IMMUNITY

Insusceptibility associated with the bactericidal properties of the blood is a later form of defense inherent in vertebrates.

Investigations have confirmed that microbes which have penetrated into the blood are rendered harmless by substances in the plasma. J. Fodor, G. Nuttall and others established the bactericidal action of blood, exudates and other fluids of animals and humans. G. Buchner showed that serum has a lethal effect on microbes, but on heating its defensive forces considerably weaken. The bactericidal matter of fresh, normal serum at first was named *alexin* (Gr. *alexein*—to ward off), then *complement* (Lat. *complementum*—means of replenishment). Since the complement dissolves some species of bacteria and cells, it is sometimes called lysin (alpha-lysin).

A substance which has bactericidal properties with regard to a number of microorganisms (causative agents of anthrax, tetanus, botulism, gas gangrene, and diphtheria, and staphylococci, pneumococci, bovine brucellae, etc.) is *beta-lysin* which is a substance of a complex nature, a thermostable fraction of normal serum, decomposing at temperatures of 63-70°C and from ultraviolet rays.

From human serum a fraction was isolated which is characterized by a bactericidal action in relation to diphtheria bacilli, and is not identical to beta-lysin.

From the blood of people with an elevated temperature a component X-lysin was isolated which dissolved mainly Gram-negative microorganisms (meningococci, paratyphoid bacteria) and to a lesser degree—Gram-positive organisms. X-lysin acts without the participation of complement, and is thermostable (withstands a temperature of 68-100°C).

Leukines, thermostable substances freed from leucocytes, per-

tain to bactericidal substances. They disintegrate at a temperature of 75-80°C. Leukins render harmless Gram-positive as well as Gram-negative bacteria.

In the serum of patients with pneumococcal diseases K. Tillet and T. Francis in 1930 discovered C-reactive protein (the name is associated with C-polysaccharide of the type II *Pneumococcus*) having immunological properties. C-reactive protein is considered to be conjugated with reactive, defensive, non-specific natural processes. It has also been found in the serum of patients with typhus fever, tuberculosis and other infections.

The component parts of urine, prostatic fluid, extracts from the liver, brain, spleen and other tissues and organs are characterized by bactericidal properties.

Under the influence of the virus the cells of affected tissues excrete interferon which does not have a specific action, but renders viruses harmless. Interferon is present in small amounts in normal human serum. *A tuberculostatic factor* has been discovered in human blood which is characterized by the ability to kill tubercle bacilli.

In 1954 L. Pillemer established that after treating serum with zymozan (obtained from yeasts) it loses its bactericidal activity. A precipitate is formed in the serum. After treating the precipitate a substance was isolated, which, on addition to serum, restored the lost bactericidal activity. This substance was named *properdin* (Lat. *perdere*—destroy). Properdin is a serum protein, an euglobulin, which plays an important part in immunity. The greatest amount is found in the blood of rats, then in a decreasing order in the blood of mice, cows, pigs, humans, rabbits, sheep and guinea pigs.

A great role in humoral activity is played by antibodies, the origin and accumulation of which occur under the influence of antigens.

ANTIGENS

The name antigens (Gr. *anti*—against, *genos*—genus) is given to organic substances of a colloid structure (proteins and different protein complexes in combination with lipids or polysaccharides) which upon injection into the body (subcutaneously, intracutaneously, cutaneously, into the mucous membranes, intramuscularly, intraperitoneally, intravenously, and orally) are capable of causing the production of antibodies and reacting specifically with them. Antigens, consequently, are characterized by the following main properties: (1) the ability to cause the production of antibodies (antigenicity) and (2) the ability to enter into an interaction with the corresponding antibodies (antigenic specificity).

Thus, antigens, on the one hand, cause a response reaction of the body accompanied by the development of certain physiological and pathophysiological processes, and, on the other hand, they

provoke the body to reconstruct itself functionally and physiologically. This is characterized by defense reactions providing for the restoration of the disturbed constancy of the body and medium.

Antigenic substances are highly molecular compounds. They have certain properties: a specific action, heterogenicity for the body, a colloid structure, and solubility in the body fluids. The breakdown of proteins to peptones, amino acids and also a deep denaturation by physicochemical effects brings about a loss of antigenic ability, while the introduction of various radicals into the protein molecule causes the loss of species specificity.

Antigenic properties are pertinent to toxins of a plant origin (ricin, robin, abrin, cortin, etc.), toxins of an animal origin (toxins of snakes, spiders, scorpions, phalangia, karakurts, bees), enzymes, native foreign proteins, various cellular elements of tissues and organs, bacteria and their toxins, rickettsiae and viruses.

Not all substances (proteins and protein complexes in combination with lipids and polysaccharides) are characterized by having antigens with similar properties. There are complete and partial antigens.

Complete antigens are substances which cause the production of antibodies in the body, and react with them in vivo as well as in vitro (foreign proteins, sera, bacteria, toxins, rickettsiae, viruses and cellular elements).

Partial antigens are known as haptenes which do not cause the production of antibodies, but can react with them. Haptenes include lipids, complex carbohydrates and other substances. The addition of proteins to haptenes even in a small amount gives them the properties of complete antigens. In this case the protein carries out the function of a conductor.

Semihaptenes (iodine, bromine, quinine, antipyrine, colloidal iron, azo dyes, azoproteins and other chemical groups) are not antigens themselves, but in combination with the proteins of the body they acquire the properties of antigens. Under the influence of these antigens immune antibodies are produced. During treatment with only one semihaptene they lose their ability to react with the antigen which caused the production of immune bodies. This reaction has been named Landstainer's inhibiting reaction.

It is well known that the properties of chemical, structural and functional specificity are inherent in all natural proteins. Proteins of different species of animals, plants, bacteria, rickettsiae and viruses can be differentiated by immunological reactions.

The antigenic function of bacteria, rickettsiae and viruses is characterized not only by species, but also by type specificity. For example, salmonellae of enteric fever cause immunity only in relation to this species of microbe, but are incapable of producing immunity against salmonellae of paratyphoid fever. Besides, inside each species of microbe there is a different amount of types which

also have specific antigenic properties. Type specificity is associated with the presence of special polysaccharide complexes in the microbial cell.

Besides species and type specificity group (generic) antigens have been revealed in closely related species. The presence of group antigens reflects the historical process of their development and genetic links.

In 1911 D. Forssman established that there are heterogenic or heterologic antigens (haptenes) found in different species of animals (guinea pigs, dogs, cats, horses, chickens, fish and turtles). If a rabbit is immunized with an emulsion from the organs of guinea pigs, then antibodies appear in the serum of the rabbit which react not only with the emulsion of these organs but also with sheep erythrocytes. Thus, in the organs of the guinea pig and sheep erythrocytes there is *a heterogenic antigen.*

It has been proven that the nonspecific properties of Forssman's heterogenic antigen are associated with the presence of lipid or polysaccharide fractions closely related in composition, which bear common properties in different species of animals, plants and microbes.

Isoantigens. Isoantigens are those substances which have antigenic properties and are contained in some individuals of a given species. They have been found in the erythrocytes of animals and man. At first it was established that in human erythrocytes there are two antigens (A and B), and in the sera—beta- and alpha-antibodies. Only heterogenic antigens and antibodies (agglutinins) can be found in human blood. These combinations may be represented as follows:

Antigens of erythrocytes (blood groups)	Serum antibodies
O	α β
A	β
B	α
AB	0

On the basis of antigenic structure the erythrocytes of all people can be subdivided into 4 groups. Consequently, variants of antigens of erythrocytes in the second (A) and fourth (AB) group were isolated. The A group consists of two subgroups—A_1 and A_2. The AB group contains antigens A_1B and A_2B, and the antigens M and N, M_2 and N_2, etc., have been revealed. At present 30 antigens are known. Besides, in the erythrocytes there is a rhesus factor (see p. 245). These data are taken into account during blood transfusion.

Autoantigens are substances capable of immunizing the body from which they are obtained. Thus, they become modified and are capable of bearing an antigenic function. These substances

include the eye lens, spermatozoids, homogenates of the seminal gland, skin, emulsions of kidneys, liver, lungs and other tissues. As under ordinary conditions they do not come in contact with the immunizing systems of the body, then antibodies are not produced against such cells and tissues. However, if these tissues are injured, then autoantigens may be absorbed, and may cause the production of antibodies which have a toxic effect on the corresponding cells. The origination of autoantigens is possible under the influence of cooling, radiation, drugs (amidopyrine, sulphonamides, preparations of gold, etc.), virus infections (virus pneumonias and mononucleosis), bacterial proteins and toxins of streptococci, staphylococci, tubercle bacilli, paraproteins, aseptic autolysis of brain tissue, and other factors. Probably, only haptenes appear, which combine with proteins and produce complete antigens capable of causing the production of antibodies.

It has been suggested that these kinds of antibodies lead to the development of glomerulonephritis, interstitial hepatitis, rheumocarditis, eye diseases and other pathological processes.

The production of autoantigens is the result of the disturbance of species specificity which provides for the antigenicity of a number of substances found in the given body. In relation to the reasons for the appearance of autoantigens, there are the following hypotheses: (1) endogenic substances become antigens only after preliminary changes; (2) endogenic substances can really be antigens and, (3) only certain endogenic substances which come from some tissues can be antigens.

In normal serum there are *cryoagglutinins* (cold haemagglutinins) in small numbers, which react with the erythrocytes of the same individual. If cryoagglutinins appear in large numbers, paroxysmal haemoglobinuria occurs.

Young venomous snakes of certain species have no antibodies and as a result of this they are sensitive to their own venom, but in adult snakes there are antibodies which neutralize their venom.

Antigenic properties of bacteria, toxins, rickettsiae and viruses, used in the practice of reproducing artificial immunity against infectious diseases, are of most practical importance.

Antigenic structure of the microbial cell. Bacteria are a complex of antigens, which include highly molecular compounds of a protein nature and biologically active specific polysaccharides.

The composition of specific polysaccharides includes different saccharic acids, amino derivatives of sugars, residue of different acids, alcohols, etc.

In 1903 S. Smith and A. Reef while studying the antigen capacity of motile and nonmotile strains of *Salmonella choleraesuis* discovered that these strains contain flagellar and somatic antigens. In 1917 E. Weil and A. Felix investigated the antigenic properties of *Proteus.* It became apparent that motile strains of this microbe on meat-

peptone agar give dense growth resembling the film appearing on glass, when it is breathed upon. They named this form the H-form (Ger. *Hauch*—breathing). The nonmotile strain upon cultivation on meat-peptone agar did not produce creeping growth and was named O-form (Ger. *ohne Hauch*—without breathing). Hence the terms *H-antigen* (flagellar) and *O-antigen* (somatic).

H-antigens are thermolabile and disintegrate at 56-80°C. O-antigens are thermostable and withstand heating up to 80-100°C.

In 1934 A. Felix and R. Pitt isolated a comparatively thermolabile antigen from virulent strains of enteric fever salmonellae which was named *Vi-antigen*.

It has been assumed that O- and Vi-antigens are arranged on the surface or at the poles of the cell. The Vi-antigen is found in the surface layer, while the O-antigen is somewhat deeper. Because of this microbes containing Vi-antigen are not agglutinated by the O-serum. In later investigations it was established that O- and Vi-antigens contain polysaccharides, lipids, and nitrogenous substances, and for this reason are included at the present time in the glucido-lipid-protein complex.

Eventually the methods of obtaining antigens free from ballast toxic substances were improved. In their investigations A. Bouaven, L. Mesrobeanu and I. Mesrobeanu received a glucido-lipid-protein complex of somatic O-antigen containing a specific polysaccharide and fatty acids by treating salmonellae of enteric fever and paratyphoids with trichloroacetic acid followed by precipitation with alcohol. By this method a polyvaccine has been prepared in the USSR, containing complete antigens of typhus fever, paratyphoid, and dysentery bacilli and cholera vibrios.

The complete somatic antigen of bacteria in the S-form contains the polysaccharide haptene which provides for species specificity, the R-forms of the same species of bacteria lose their specific polysaccharide, and do not have a marked species specificity. All of these features of antigen structure are of great practical importance, and are taken into account in serological diagnosis of infectious diseases and in the manufacture of vaccines and sera.

Recently *protective antigens* have been revealed, which are absent from the microbial cell, but which occur during bacterial multiplication in the tissues of the body. Protective antigens have been found in exudates of animals suffering from anthrax. They can be obtained by cultivating anthrax bacilli on live tissues and in special nutrient media composed of amino acids.

Protective antigens have considerable protective properties and can be used in the practice of immunization against some infectious diseases, in particular anthrax and plague. They, probably, pertain to substances of a protein nature and to thermolabile proteins. Similar substances were discovered in the causative agents of whooping cough, brucellosis and tularaemia.

The presence of antigens in nonpathogenic bacteria has been established, which are inherent in pathogenic bacteria and are capable of causing the production of antibodies against them.

Microbe toxins also have antigenic properties. Rendered harmless by formalin and heat treatment, exotoxins lose their toxic properties and almost completely retain their antigenic functions. They are known as anatoxins, and are widely used in immunizing people against diphtheria and tetanus and for hyperimmunization of horses in order to obtain antitoxic therapeutic sera against diphtheria, tetanus, botulism, gas gangrene, etc.

Rickettsial antigens. In order to obtain rickettsial antigens a suspension of rickettsiae is used from a culture of the membranes of a chick embryo, from the guts of body lice or from the lungs of white mice infected with rickettsiae. To free rickettsiae from foreign protein matter and tissue components, they are treated with ether or are differentially centrifuged. In chemical composition rickettsiae pertain to liponucleoproteins and have the properties of complex antigens. They are characterized by species and type specificity which allows the differentiation of the species and type specificity of rickettsiae according to known specific sera, and by means of known antigens the corresponding antibodies in the sera of animals and man can be determined.

Antigenic structure of viruses. All known viruses are characterized by antigenicity and a specific action. Their antigenic structure varies: in some groups it is complex, in others—simple. They have species and type specificity. With the help of immune reactions, types have been revealed in some viruses. For example, the virus of influenza consists of types A (A1, A2), B, C, and the virus of poliomyelitis of three types, I, II, III.

Modern methods of specific serotherapy and seroprophylaxis of virus diseases (measles, influenza, etc.) and also serodiagnostics (complement-fixation reaction, virus neutralization test, and haemagglutination-inhibition reaction) are based on this principle.

Upon the destruction of some viruses soluble antigens are liberated, which have the properties of toxins (influenza virus). The smaller viruses are nucleoproteins and when their particles are disintegrated soluble antigens are not liberated (viruses of yellow fever, poliomyelitis, foot-and-mouth disease). Some viruses (phages, vaccinia virus) have two and more antigens.

ANTIBODIES

Antigenic substances which penetrate the body disturb the constancy of the composition of blood. Antibodies are synthesized under their influence.

Antibodies are specific substances produced in the bodies of ver-

tebrates upon the introduction of antigens, and are capable of a specific association with them.

As a result of an obvious or latent infectious disease antibodies appear in the sera of animals and man.

Antibodies appear not only as a result of an infection, but also due to immunization by live (attenuated) or dead bacteria, rickettsiae, viruses, toxins and other antigenic substances.

Antibodies which occur under the influence of active immunization are named immune antibodies in contrast to normal antibodies which are often found in the sera of man and animals who have not had infectious diseases and who have not been exposed to immunization.

Normal as well as immune antibodies are capable of rendering harmless the causative agents of infectious diseases in various ways.

The nature of antibodies. At present it has been established that antibodies are globulins which have been altered under the influence of antigens.

Antibodies of one and the same antigen formed in the blood of different species of animals, like the corresponding normal globulins, differ in chemical composition. Molecules of antibodies, like normal serum globulins, are probably asymmetrical. It has been established by the method of fractionation that antibodies are found in globulin fractions.

The transformation of normal globulin into immune globulins is accompanied by an alteration in the spatial configuration of active atomic groups of the protein molecules. A change in protein metabolism lies at the basis of this process.

Antibodies are not homogenous. There are many varieties of antibodies which have different physicochemical properties. As to the mechanism of the production of antibodies there is still no unanimous opinion, although several theories have been propounded.

From the earliest works an attempt was made to explain the formation of antibodies as being due to the transformation of antigens. In 1893 H. Buchner assumed that antigens transform into antibodies, and G. Smirnov (1896) and other authors adhered to this point of view. They affirmed that they obtained the antibodies while heating a mixture of antigen and animal serum or by passing an electric current through it. However, the substances obtained did not have the specificity inherent to antibodies. In spite of the fact that this conception was not recognized, in 1930 W. Manwaring affirmed that the formation of antibodies takes place as a result of the parenteral digestion of the antigen under the influence of synthetic enzymes. V. Zdravosmyslov (1926-1927) and G. Ramon (1936) adhered to an analogous point of view. According to the latter, the determinant group is transformed into the active group of the antibody, etc. In 1940 P. Jordan assumed that the determinant group of antigen combines with proteins and produces autocatalystic multiplication of the antibody molecules.

The second group of theories suggests that the mechanism of antibody synthesis is due to selection at a molecular and cellular level. Ehrlich's side-chain theory (1906) pertains to the theories of selection at a molecular level, and in spite of severe criticism in recent years this theory has again attracted attention

due to the development of the study of selection and genetics and microbiology and immunology.

According to Ehrlich's theory, cells have a central nucleus with a functional specificity, and side chains or receptors which induce the process of nutrition. Under the effect of bacterial antigens, their toxins, rickettsiae, viruses and other substances the receptors are cast off from the cells and are replaced according to the law of hypercompensation by a large amount of other receptors (antibodies). The latter are overstocked on this site, are cast off from the cell, and in a free state enter the blood where they react specifically with the corresponding antigens. All antibodies which were produced were subdivided into three categories: (1) first order receptors, (2) second order receptors and (3) third order receptors (Fig. 59). P. Ehrlich considered the formation of antibodies to be an overproduction of something pre-existing in the cell and thus genetically determined.

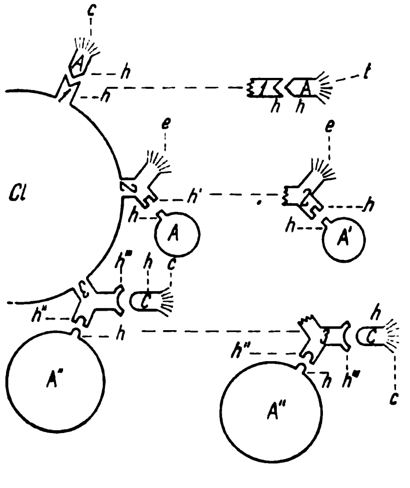


Fig. 59. Diagram of antibody formation according to Ehrlich

Cl—cell; 1—first order receptor with haptophore group h; 2—second order receptor with haptophore group h′ and ergophore group e; 3—third order receptor with two haptophore groups: cytophilic h″ and complement h‴, A, A′, A″—various types of antigens; t—toxophore group; C—complement with haptophore group h and ergophore group C′

A development of Ehrlich's ideas is the theory of selection of globulin molecules of Yuernet (1955), which suggests that the antigen makes a selection among an unlimited amount of pre-existing configurations of gamma-globulin, and stimulates the synthesis of molecules of globulin with the same supplementing configuration. According to this theory, specific antibodies are formed according to preformed models which are quite often present in small undiscernible amounts among populations of gamma-globulin molecules found in normal serum. The action of the antigen in this case is not to elicit changes in the configuration of globulin, but to select for differential proliferation those pre-existing models which have the corresponding configuration.

The next stage of development of Ehrlich's ideas was the clone-selection theory of Burnet which at present arouses most interest. According to this theory, immunity and synthesis of antibodies are the function of mesenchymal cells. Each clone is capable of reacting immunologically with a very small number of antigenic determinants. Due to the contact of the cells with the specific antigen structure a change in the number and types of mesenchymal cells of the body takes place. The interaction of the cells of the body with the antigen may be accompanied by (1) the death of or injury to the cells with the release of products causing a stimulating or harmful action; (2) the stimulation of proliferation with or without morphological changes and (3) the transformation of cells into plasmatic cells capable of active synthesis and release of the antibodies.

According to Burnet, each lymphocyte, plasmatic cell or macrophage bears an immunological mark. In one lymphoid cell there may be about 10,000 specific receptors against any possible determinants. In the embryonic period 5,000 of these receptors are inhibited on contact with their own body structures.

Burnet's theory merely implies that the population of lymphocytes in the body is heterogeneous and consists of a large number of different clones. Different clones produce antibodies which react with various antigens. The clones of lymphocytes arise by mutation, and the antigen serves as a factor of selection. The first part of the clone-selection theory has been confirmed experimentally. It has been proved that separate cells produce only one type of antibody. As to the second part of the theory on the pre-existence of cells producing different types of antibodies, there are serious objections since the assumptions on the pre-existence of cells, like the pre-existence of Ehrlich's side-receptors, are speculative.

The above mentioned viewpoints on the formation of antibodies are opposed to the conceptions at the basis of which are laid the mechanisms associated with the change in protein synthesis. In 1928 N. Gamaleia stated the basis of the most widespread theory, according to which receptors are not preceded in the cell, but are formed in it as a result of a reaction to irritation caused by an antigen. An antigen which has passed through the cell leaves a trace on it similar to an impression made in sealing wax. The cell subsequently begins to cast off these impressions (antibodies), which have an affinity to the antigen provoking them. This theory is known as the theory of impressions. Later F. Breinl and F. Haurowitz (1930) stated the hypothesis according to which antibodies represent altered globulins formed by a direct influence of the introduced antigen in the body. The molecules of antibodies have a spatial configuration different to that of the normal globulin (Fig. 60).

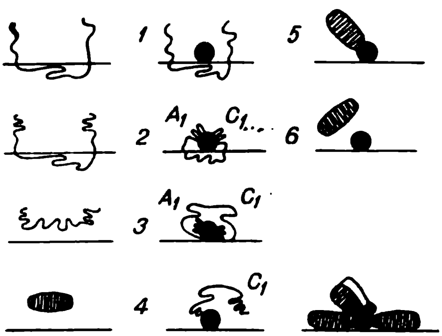


Fig. 60. Synthesis of antibodies

1-6—stages of synthesis

According to the Haurowitz-Pauling theory, the molecules of the antigen act as a tough template which serves to produce numerous negative impressions on the formed molecules of the globulin antibodies.

In 1949 F. Burnet and F. Fenner created the "indirect template" theory of the formation of antibodies in which an attempt was made to explain the reason for the synthesis of antibodies after the introduction of the antigen. According to this theory the antigen, having penetrated the cell of the embryo type, elicits in its genetic nuclear apparatus hereditarily fixed changes, with which the newly acquired ability of the cell to synthesize specific globulins is associated.

Investigations carried out with the help of radioactive isotopes showed that synthesis of antibodies is an independent process which does not depend on the synthesis of normal globulins, and preparatory induction precedes the synthesis of antibodies (W. Taliaferro, L. Taliaferro, D. Tolmedge, G. Gurvich, et al). By means of isotopic techniques, it has been established that antibodies are not

produced by an alteration of the molecule of the already formed gamma-globulin, but are synthesized from amino acids (H. Green, H. Anker).

A forcible objection to the theory of impressions is that antibodies are formed even after the complete disappearance of antigens from the body.

According to Sevag's theory (1945), antigen serves as a catalyser causing the synthesis of antibodies in an amount considerably greater than the number of antigens used up.

According to L. Pauling, gamma-globulins are synthesized first of all as a polypeptide chain and have no immunological properties. They are formed in the process of second or tertiary clotting of a long thin filament into a spherical or cigar-shaped molecule of antibody.

F. Carouche considers that firstly a plastic framework is formed which can accept a spatially complementary structure upon contact with the antigen. After completing "the packing" the coiled chain is stabilized in the fixed framework due to the locking of disulphide and hydrogen ties between the neighbouring fragments, after which the antigen template is freed and possibly is used again.

The problems concerning the specificity of antibodies can be solved with the help of chromatography and electrophoresis. It is possible that each type of antibody has its own original sequence of amino acids. Then the information needed for the building of these antibodies should be coded in the genetic apparatus of the cell, which produces antibodies.

Thus the current theories on the formation of antibodies differ in accordance with the following questions—whether the immunological reaction to foreign antigen determinants leads to the formation of imprints on mesenchymal cells, or this process is a response reaction partially prepared for subsequent mutation and selective proliferation of the corresponding clones synthesizing antibodies.

In chemical composition and physical properties antibodies do not differ greatly from normal globulins; they have almost similar isoelectric points, viscosity, molecular weight (from 160,000 to 1,000,000), and sensitivity to the action of temperature and various other denaturing factors.

Antibodies are thermolabile substances, and are denatured upon heating to 70°C for one hour. The pH of the medium and also other factors which have profound effects on proteins influence the activity of antibodies. It has been established that antibodies are not denatured by precipitation with ethyl alcohol at a low temperature (from 0 to +4°C), but are denatured by alcohol at high temperatures. Neutral salts (magnesium sulphate, ammonium sulphate and sodium sulphate) cause a precipitation of proteins, but do not denature antibodies. Because of this ethyl alcohol at a low temperature, and magnesium, sodium and ammonium sulphates are used for fractionation of immune sera and for obtaining them in a pure state.

By means of electrophoresis A. Tiselius separated serum proteins, according to their mobility in an electric field, into albumins and three globulin fractions, α, β and γ, while in immune sera a new fraction was found—T or β_2 located between the β- and γ-fractions.

At present electrophoresis is carried out by various methods—optic and zonal electrophoresis on a gel and on paper. Electrophoregrams of normal, antibacterial and antitoxic sera are shown in Fig. 61.

Gamma-globulins are the protein fractions which possess the lowest electrophoretic mobility and consist of proteins related in properties.

Recently a new method has been introduced for the investigation of toxins, anatoxins and immune sera, *immunoelectrophoresis*, which permits a more accurate characterization of the antigen and antibody complexes.

Site of formation of antibodies. E. Metchnikoff assumed that the elements of organs which produce phagocytes (the spleen, bone

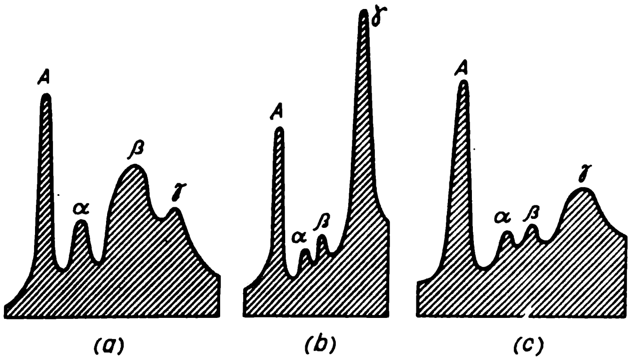


Fig. 61. Electrophoregrams of normal (*a*), antibacterial (*b*) and antitoxic (*c*) sera

A—albumins; α, β, γ—globulins

marrow, lymph nodes) or the phagocytes themselves excrete protective substances and specific factors which pass into the plasma. Antibodies are produced in the lymphoid tissue, i.e., in those organs in which serum globulins are synthesized: in the spleen, lymph nodes, liver, vascular endothelium and bone marrow. It is presumed that antibodies can be formed by cells of other tissues and organs. The plasmatic cells are of most importance in the formation of antibodies from the group of free cells of the connective tissue.

The production of antibodies takes place in two phases: specific and nonspecific. The specific phase of production of antibodies is due to the direct action of the antigen on the cell of the lymphoid tissue, and is accompanied by intracellular synthesis of globulin. The nonspecific phase is characterized by the liberation of antibodies which may take place reflexively under the influence of nonspecific stimulants.

After the introduction of an antigen antibodies do not appear immediately, but only after a certain length of time. This phase of immunogenesis during which antibodies are not found in the body is known as the induced phase. After this the phase of production

and liberation of antibodies ensues. Their concentration in the blood increases, reaches a maximal level and then slowly diminishes.

In the immunized body as well as in the body which had suffered from an infectious disease, but which had lost the ability to retain antibodies the blood serum antibody titre is increased under the influence of specific and nonspecific stimulants. This phenomenon is known as *trace* or *anamnestic reaction*. This property of the body to strengthen its immunological ability is the result of a long historical process of the formation of complex defense adaptations which manifested themselves under the influence of the action of the central nervous system. These peculiarities of potential immunity explain to a certain degree the comparatively rare relapses of diseases in many infections.

The studied immunological laws have provided for a wide employment of the method of revaccination involving repeated injections of antigens, in the practice of immunizing people against infectious diseases and hyperimmunization of large animals for obtaining therapeutic sera. This method provides for the increase in immunological activity.

There are cases of congenital inability of the body to synthesize gamma-globulins (*agammaglobulinaemia*), while the degree of susceptibility to the infection is directly proportional to the decrease of the gamma-globulin level, and the decrease in production of the necessary amount of antibodies in the blood. In other cases the percentage of gamma-globulin in the blood is not lower than normal, but the ability to produce antibodies against a particular antigen is lacking. Sometimes the decrease of the gamma-globulin level is transitional as can be observed in children after birth, when the amount of gamma-globulin gradually declines and at the age of 2-4 months remains at a low level.

At present the phenomenon of agammaglobulinaemia or hypogammaglobulinaemia is known as the antibody deficiency syndrome. People with this syndrome are often exposed to repeated severe bacterial infections. There are specific and nonspecific gammaglobulins. The immune function is associated with the former. Nonspecific gamma-globulin is not capable of causing an immune reaction.

There are three forms of antibody deficiency syndrome.

1. *Congenital form* in which the antibody deficiency syndrome is the main affection. It is observed in newborn and small children as sporadic cases or is characteristic of the whole family (congenital and acquired forms) in youths and adults. In the light of genetical data this form is regarded as a recessive defect of protein metabolism associated with sex. It is possible that it is due to a congenital deficiency in the lymphoid apparatus.

2. *Acquired form* in which the antibody deficiency syndrome does not depend on the sex or age, but is associated with various diseases

of children. It is frequently found during affections of the lymphoid tissue and less frequently during affection of other tissues and also during endocrine diseases, metabolic diseases, nephrosis, etc.

3. *The transitional form* manifests itself only in early childhood in children of both sexes, during which there is probably a more marked deficiency in the synthesis of normal gamma-globulin and antibodies.

The body of the child does not produce gamma-globulin in the first weeks, while maternal gamma-globulins disappear to the 9th month of life.

Immunological paralysis ensues when an excess of antigens is introduced into the body. The body loses its ability to be immunized by deliberate vaccinating doses. The mechanism of this phenomenon has not been fully explained. It has been suggested that immunological paralysis is caused by a binding of the antibody with the antigen which is retained for a long period in the body. Then a blockade of the reticulo-endothelial system by the polysaccharide antigen takes place.

A great influence is caused by many other factors (nutrition, vitamins, ionizing radiation, hormone production, cooling, overheating and intoxication) on the production of antibodies. During starvation or insufficient protein nutrition the production of antibodies is diminished. The state of vitamin deficiency also inhibits the synthesis of antibodies. Ionizing radiation inhibits the production of antibodies, if the body is exposed to irradiation prior to the injection of antigen. Immunization directly after irradiation does not lower the antibody titre. Cells in the induced phase of antibody production (i.e., in the period of antigen fixation by the cells) are most sensitive to the action of ionizing radiation. The state of stress (see p. 223) provides for a sharp drop in the over-all resistance of the body including humoral immunity. The production of antibodies is decreased under the influence of antibiotics administered for the treatment of patients in the early stages of disease. Thus, of certain significance for maximal development of immunity are chemical composition, physicochemical properties, conditions of administration, intervals and doses of antigen, state of the body and influence of the external environment.

In the past immunity has been regarded as an accumulation of antibodies the role of which was to render harmless pathogenic microorganisms and their toxins. The first to oppose this simplification of the mechanism of humoral activity was E. Metchnikoff, who affirmed that insusceptibility created as a result of active immunization is a complex phenomenon, and pointed to the importance in this process of changes in the sensitivity of the body.

It is considered that the production of antibodies is not a defense adaptation, but merely expresses the ability of the body to carry out the synthesis of globulins.

It is known, for example, that during rheumatic fever the interaction of the antibody and antigen leads to the damage of the interstitial substance of the connective tissue, the liberation of histamine and the development of an inflammatory reaction.

During multiplication of viruses of poliomyelitis, tick-borne encephalitis, some strains of herpes viruses, etc., in the nerve cells, autoantigens which provoke synthesis of antibodies are produced. The reaction of these antibodies with autoantigens causes profound damage to the nervous tissue.

Autoantibodies against kidney tissue have been revealed in glomerulonephritis, malignant nephrosclerosis, of septic endocarditis, against liver cells in infectious hepatitis and against myocardial cells in rheumatic fever. Of an autoimmune nature are diseases such as some haemolytic anaemias and thrombocytopoenia due to heat and cold autoantibodies, Hashimoto's disease, rheumatoid arthritis, systemic lupus erythematosus (see p. 256), possibly periarteritis nodosa, sympathetic ophthalmia, vascular purpura, noninfectious encephalitis, chronic pancreatitis, nonspecific ulcerative colitis, etc.

Undoubtedly anaphylactic shock, serum sickness and allergic diseases are due to injury of the cells by the antigen-antibody complex.

The production of immunity depends on the degree of antigen stimulants taking part in the production of antibodies and other defense reactions. In frequent injections of the antigen or when it is injected in large doses *immunity inhibition* may occur during which the body will not respond to the action of antigen by further production of immunity. During simultaneous injection of strong and weak antigens into the body the stronger antigen may inhibit the weaker immunological process.

Antigenic action on the body in the embryonic period changes the immunological condition. The body fails to produce antibodies against the antigen with which it came into contact in the embryonic period. This state is named *tolerance* (Lat. *tolerantia*—endurance). Tolerance is the ability of the body to accept for itself foreign material and not respond to it by immune reactions, if this antigen was injected in the embryonic period or a short time after birth. Tolerance can be reproduced as a result of embryonic parabiosis, tissue transplantations and injection of antigen material into the embryo. After birth tolerance may be acquired as a result of subcutaneous, intraperitoneal and intravenous injections of the antigen. It can be reproduced by completely substituting the blood of the newborn animal with the blood of a homologous donor. Immunological tolerance is not inherited tolerance and to a certain extent resembles the phenomenon of immunological paralysis. Upon coming into contact with the antigen in the embryonic period of the cell the produced antibodies either do not reproduce or completely disintegrate. Immunological tolerance explains the failure of the

body to produce antibodies to its own antigens. It is possible that the phenomenon of agammaglobulinaemia also to a certain extent depends on tolerance.

Immune bodies, as has been stated, are not the main reason for acquired insusceptibility, however, the therapeutic and prophylactic significance of immune sera has been indisputably approved for more than 65 years. That is why humoral reactions of immunity are of great practical significance.

The ability to produce humoral immunity as a form of defense of a later origin is more absolute than phagocytosis and other defense reactions of tissues. Humoral immunity is inherent only in vertebrates; plants do not produce antibodies, as a result of which viral diseases proceed throughout the life of the plant at first in the acute and then in the chronic form.

As noticed in this short review, tissue and humoral immunity plays a great part in the general complex of the mechanism of insusceptibility. The long-term application of vaccines and sera has shown that in some cases (smallpox, tularaemia, rabies) specific prophylaxis is the decisive link in antiepidemic measures. Seroprophylaxis and serotherapy in measles, tetanus, diphtheria, botulism, anthrax have had a great influence on lowering the fatality. Widespread use of gamma-globulin for prophylaxis of measles, scarlet fever, whooping cough, rabies, poliomyelitis, epidemic hepatitis, and other diseases also gives evidence for the indisputable effectiveness of antibodies.

In spite of the great part played by tissue and humoral immunity the state of immunity is not exhausted by these forms of defense. Immunity may reveal itself without the participation of phagocytosis and antibodies. It has been noticed that children suffering from agammaglobulinaemia comparatively easily tolerate measles, recover and do not become infected again. The majority react normally to vaccinations against smallpox. Consequently, immunity may be reproduced without antigens, and the presence of antigens is not always an index of immunity. In some cases the high level of antibodies does not protect the body from disease. The phenomena of incomplete phagocytosis and prolonged carrying of pathogenic bacteria suggest that these forms of immunity fail to provide for the complex process of insusceptibility which takes place in higher animals and man. A new form of defense has been revealed which is directed at restoring the impaired functions of the body.

FUNCTIONAL IMMUNITY

Russian microbiologists (V. Barykin, I. Krichevsky, etc.) already during the early stages of development of the study of immunity investigated defense reactions not isolated, but in a complex and in interrelation with all the systems of the body.

At present it has been established that in higher animals and man the nervous system is the apparatus for new mechanisms of immunity not associated with phagocytosis or with antibodies, which I. P. Pavlov named "the physiological measure" of the body against disease.

In many infectious diseases disturbances in the circulation of the blood occur due to the loss of arteriole tone in some cases, and as a result of a sharp drop in blood pressure toxinfectious collapse may ensue. On stimulating the circulatory system with antigens a rise in blood pressure takes place which prevents the development of collapse. Reflex increase in the blood pressure is the manifestation of functional immunity directed not at the microbes or their toxins, but at restoring the impaired tone of the blood vessels. Consequently, the body is capable of self-regulating processes caused by antigens.

Microbes and their toxins have an influence on heat exchange and cause fever. The rise in body temperature lethally acts on antigens by causing a considerable increase in the oxidative processes by means of which they are destroyed.

Of great importance in defense reactions of the macroorganism is the strengthening of the excretory functions of the stomach, intestine, upper respiratory tract, kidneys and other organs. In the strengthening of the excretory function antigens manifest their influence on the neuroreflex regulation.

Antigens as stimulants alter the nature of metabolism. In the process of development of infection or intoxication the production of acetylcholine in the brain, liver, intestine, spleen and other organs is increased. Its transformation from the bound to the free state leads to the functional disorders of cardiac activity, an increase of glandular secretion of the digestive tract in convalescents, and an increase in the production of antibodies. Under the influence of antigenic stimulants the activity of cholinesterase is increased in the blood and tissues of the immune animal, which inhibits the intoxication with acetylcholine freed from the tissues. It has been established that in the process of the formation of immunity there is an increase in the amount of adenyl-polyphosphoric compounds and an acceleration in the conversion of adenosine diphosphoric acid into adenosine triphosphoric acid. A rise in tissue respiration is also observed.

All of these processes are specific. They occur and are activated under the influence of antigens with the aid of which immunization of the animal was carried out.

Functional immunity includes defense-adaptative mechanisms studied by G. Selye and named stress, which signify the origin of one type nonspecific reactions produced under the influence of different injuries to the body (intoxications, infections, burns, traumas, cooling, etc.).

Physical, chemical, pharmacological and biological factors which elicit the state of stress were named stressors by G. Selye. Stressors act on the hypophysis humorally as well as through the nervous system. Stressor agents include cold, heat, ultraviolet and ionizing radiation, pathogenic microorganisms, their toxins and other factors capable of provoking stimulation in the body.

An adaptation syndrome may be general or local. It is provided for by the action of the hypophyseal-adrenocortical system which is functionally bound with the hypothalamic centre. Under the influence of a general or local stressor, the hypophysis begins to intensively secrete the adrenocorticotrophic hormone (ACTH), stimulating the function of the adrenal glands and causing an intense secretion of the anti-inflammatory hormone of the cortisone type, which lowers the reactivity of the connective tissue. The hypophysis also secretes the somatotrophic (STH) growth hormone which on the contrary causes an increase in the reactivity of the connective tissue (Fig. 62).

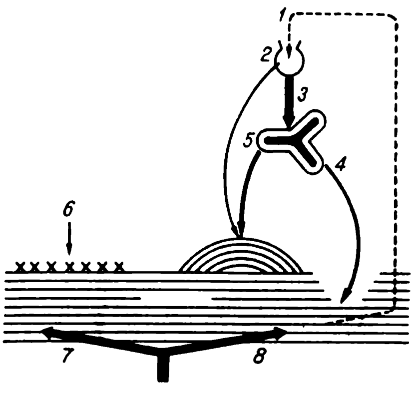


Fig. 62. Diagram of the mechanism of stress action

1—regulating action of the hypothalamus; *2*—hypophysis secreting STH (somatotrophic hormone); *3*—secretion of ACTH (adrenocorticotrophic hormone) by the hypophysis; *4*—secretion of anti-inflammatory hormones by the adrenals; *5*—secretion of adrenal hormones reinforcing the inflammatory reaction; *6*—specific reaction of the body; *7*—specific action of stress; *8*—nonspecific action of stress

The adaptation syndrome proceeds in three stages: (1) *the alarm reaction* which is the sum of the phenomena developing successively in the nonadapted organism under the influence of a stressor and leading to a fatal condition or to a state of stability; (2) *the stage of resistance* characterized by an increase of the resistance to the action of the stressor with the disappearance of most morphological and biochemical changes; (3) *the stage of exhaustion* developing as a result of the long, continuous, extreme action of the stressor with the loss of the adaptation acquired in the stage of resistance.

Because of the definite role of the hormone system in defense reactions during epidemic hepatitis, parotitis, meningoencephalitis, miliary tuberculosis and its exudative forms, tuberculous meningitis the use of corticosteroids is recommended.

At present there is no doubt as to the existence of *areactive immunity* under the influence of which various complex changes take place directed at maintaining the normal level of the circulation of the blood, respiration, heat exchange and other functions of the immune

organism. Areactive immunity manifests itself as protective inhibition in the cerebral cortex preventing disintegration and exhaustion of its tissues from stimulants of unusual strength.

Many scientists have established that under the influence of specific antigens and nonspecific substances in the body resistance is quite rapidly produced to some pathogenic bacteria and their toxins. This kind of reproduction of homologous and heterogenous insusceptibility has been named *tachyphylaxis* (quick protection) or *proimmunity*. Tachyphylaxis arises within several hours and lasts from 3 to 10 days. In most cases it is reproduced in relation to toxic substances and weakly virulent bacteria. Some investigators regard these forms of immunity as a result of an increased state of defense of nonspecific resistance produced by the lowering of the reactivity of tissue cells in response to the stimulant. The rapid development of resistance in animals to bacterial toxins under the influence of a specific antigen develops without the production of antibodies (antitoxins). Others presume that rapidly reproducing resistance to toxins as a result of the injection of large doses of anatoxin ensues, at first, due to the areactivity of tissues and then as a result of the production of antibodies. It is possible that in the given mechanism of susceptibility the blockade of sensitive tissue cells due to the injection of large doses of anatoxin is of certain importance.

These examples do not exhaust the diversity of functional immunity.

In the defense reactions of the macroorganism a definite role is played by *bacterial interference*. It has been established that upon infection of animals with brucellae insusceptibility is developed to anthrax bacilli, while antibodies are not produced to anthrax bacilli. Interference has been confirmed between brucellae and tularaemia bacteria, tubercle bacilli and causative agents of plague, and tubercle bacilli and anthrax bacilli. It is possible that during bacterial interference, a blocking occurs of the most sensitive tissue cells, which creates unfavourable conditions for one of the microbes. In immunity during viral diseases a great role is played by *viral interference* the mechanism of which will be explained later.

Normal microflora has a definite significance in immunity which in some cases manifests itself in relation to pathogenic microbes as antagonists, and in others as synergists.

* * *

Depending on which agents the defense forces of the macroorganism are directed against immunity is subdivided into antibacterial and antitoxic, antiviral and antiparasitic immunities. This division of immunity by no means excludes the unity of all defense reactions and their interrelations. Absolutely autonomous forms of immunity do not exist, but all of them are interrelated and

manifest their protective action in the entire organism with all systems taking part. During some infectious diseases antibacterial immunity occurs to a more marked degree, while in others—antitoxic immunity occurs. This differential manifestation of immunity is determined by biological peculiarities of the causative agent and by defense reactions which were formed in the process of evolution of the micro- and macroorganisms.

Antibacterial immunity. During an active manifestation of the defensive forces of the body a typical clinical picture of the disease does not ensue, and infection does not lead to disease in the human. In the blood and the reticulo-endothelial system microbes are exposed to the action of cellular and humoral factors.

Sterile and nonsterile (infection) immunity. Sterile immunity is that type of immunity in which the body has completely freed itself of the causative agent. Immunity after measles, whooping cough, cholera, smallpox, etc., is an example of this kind of immunity.

During brucellosis, tuberculosis, leprosy, syphilis and other diseases of long duration the relative immunity coincides within a definite length of time with the presence in the body of causative agents of infectious diseases. This type of immunity is named nonsterile (infection, *depression*) immunity. During chronic diseases it has been possible to reveal for a long time the simultaneous presence in the body of the causative agent and relative immunity to repeated infection or to exacerbation of the existing infection.

Specific defense reactions originate and develop simultaneously with the infectious process. Thus, during the stage of nonsterile immunity of different duration the development of all defense reactions without exception takes place, terminating in the formation of sterile immunity. Thus, at first, infection and then post-infection immunity develops, and the phase of nonsterile immunity is replaced by the phase of sterile immunity. This general conformity is inherent to the infectious diseases of long duration (chronic), i.e., malaria, brucellosis, tuberculosis, etc.

Antitoxic immunity. In diseases the causative agents of which produce exotoxins certain tissues and organs are selectively infected. In the process of evolution of defense reactions the body has developed the ability to render harmless not only the microbes, but their toxins. Toxins are rendered harmless mainly by neutralizing them with antitoxins.

In the practice of immunization against diphtheria and tetanus antitoxic immunity is reproduced by introducing anatoxins, and specific treatment of patients with diphtheria, tetanus, botulism and gas gangrene is carried out with the corresponding antitoxic sera.

However, antitoxic immunity should not be reduced only to the reaction of neutralization. During infection with toxigenic microbes

the body responds by producing defense mechanisms directed at the toxin and the causative agent.

Antiviral immunity. In many virus diseases (measles, German measles, chickenpox, yellow fever, smallpox, parotitis, etc.) a sound immunity is built up, sometimes for life. Only after a few viral infections (viral cold, dengue, pappataci fever) is a weak immunity produced.

Many scientists have established that leucocytes and the cells of the reticulo-endothelial system do not play any essential part in rendering viruses harmless, which are obligate intracellular parasites.

The most important defense mechanism is considered to be the pyrogenic action of factors capable of causing fever. In the absence of cells sensitive to the virus the causative agents which have invaded the body perish.

Of great significance is immunity associated with *the excretory functions* of the body. In a series of virus diseases it has been established that viruses can be expelled through the upper respiratory tract, intestine and kidneys.

In immunity to virus diseases of certain significance are thermostable and thermolabile serum inhibitors, and also antibodies capable of neutralizing the virus before it becomes bound to sensitive cells. Antibodies, as a rule, are not capable of neutralizing infectivity of viral nucleic acids.

Some viruses upon infection with tissue cultures are rendered harmless by antibodies, but others are not and can cause a spread of infection. During acute viral infections accompanied by a cytopathogenic effect, antibodies provide a protective action. During the latent form of infection they do not have any influence on the development of the infection caused by the same virus. The neutral complex (virus-antibody) can be reactivated by dilution, changes in pH and other factors. The virus can be reactivated from the superneutralized complex under the effect of freon.

Humoral immunity includes the ability of some cells of the macroorganism to secrete adaptive enzymes (desoxyribonuclease, ribonuclease) capable of destroying the corresponding viral nucleic acids.

Antiviral immunity may be revealed as a result of interference (mutual weakening) of viruses. The mechanism of this defense depends not on antibodies, but on the ability of one virus to neutralize the action of the other by blocking the sensitive cells.

The principle of interference has not been revealed. It has been suggested that among interfering viruses there is a competition for substrates in the host cells which they utilize for their life activities.

It is possible that during interference the genetic determinant system (DNA or RNA) of one virus hinders the manifestation of the genetic system of the other virus.

Interference takes place among definite strains of one and the same species of virus. Thus, for example, the vaccine strains against rabies, influenza, and yellow fever have interfering properties relative to the causative agents of these diseases. The phenomenon of interference between viruses affecting plants, animals and humans has been established. This incompatibility was revealed between viruses of rabies and foot-and-mouth disease, between viruses of rabies, vaccinia and herpes, between viruses of poliomyelitis and lymphocytic choriomeningitis.

The manifestation of antagonism between viruses is of great practical importance, as it can be used for the specific prophylaxis of viral diseases.

Under the influence of the virus some cells become resistant to repeated infection by the same virus. It has been noticed that in a nutrient medium of a tissue culture a substance appears to be capable of neutralizing the virus. This kind of immunity does not have a strictly marked specificity. As has been established, it is provided for by the presence in the blood of interferon which is a protein with a molecular weight of 63,000. It is resistant to low temperatures and to ultraviolet rays, does not lose its activity at a pH from 2 to 10, does not have antigenic properties, and is nontoxic. Interferon produced under the influence of DNA and RNA of one virus is active against a number of other viruses. In a typical viral disease within one or two days the cells may secrete such an amount of interferon that it is capable of arresting the infectious process. Interferon has the properties of an antibiotic with a wide spectrum of action. The defense role of interferon is especially well expressed during viral infections transmitted by droplets, while in those infections which are transmitted by blood-sucking insects its action is inferior to that of antibodies. It acts, as had been presumed, by bringing about a decrease in ATP formation, which is sufficient for the cell requirements, but insufficient for the synthesis of the virus. It is possible that interferon inhibits the genetic information of foreign nucleic acids.

Immunity to tumours of a viral origin (sarcoma, leukosis, avian lymphomatosis, skin and mouth papilloma in rabbits, papillomas in cattle and other animals, leukaemia and breast cancer in mice, kidney cancer in frogs, warts and condillomas in man) is similar to immunity to viral infections. The difference is that in tumours of a viral origin there are two antigens: one is the viral substance, while the other originates in the tissue as a result of transformation of the normal cell into a tumour. These antigens stimulate the production of corresponding antibodies. However, the immunological processes of tumours are different to those which occur during viral diseases. This is explained by the fact that tumour viruses do not cause the destruction of cells of the invaded tissue, but bring about their transformation into tumours. That is why immunity to tumours

is more complex. Tumours give rise to autoantigens and the corresponding antibodies. The practical use of immunity during tumour formation is in the experimental stage of investigation.

Thus, antiviral immunity as a self-regulating system of cells and humoral defense mechanisms is subordinated in all of its main properties to general conformities, and at the same time is characterized by specific peculiarities.

Antiparasitic immunity. Insusceptibility to pathogenic parasites is characterized by a variety of mechanisms. The production of immunity depends on the character of the localization of the parasite. Some of them localize in the tissues (trypanosomas, leishmaniae, malarial plasmodia), others in the lumen of the intestine (*Entamoeba histolytica*), and others in the lumen of the intestine and in the tissues (intestinal balantidia, coccidia, helminths).

Antiparasitic immunity is brought about by the defense action of antibodies and an increased activity of phagocytes. Under the influence of antibodies the life processes in parasites are deeply disturbed, and then the parasites are dissolved. Phagocytes under the influence of opsonins absorb and digest minute parasites, while large parasites are immobilized in the tissues by the mutual action of many cells.

Usually at first an active effect is manifested by the microphages, then the macrophage which has a more effective action enters into combat with the parasite. The number of defense cells greatly increases and in some cases is an index of the infection of the body by one or another species of parasite.

In spite of the common character of natural immune reactions during parasitic and bacterial infections the problem of prophylactic vaccination against diseases caused by parasites has not yet been worked out. Only in relation to skin leishmaniasis (Borovsky's disease) N. Latyshev received a vaccine used in endemic foci.

Community immunity. Besides the above mentioned forms of immunity there is the concept of community immunity (group, focal). This kind of immunity is created as a result of having had obvious or latent diseases, and also under the influence of a carrier state in definite foci of epidemic outbursts. In most cases community immunity is post-infectious. In places with high morbidity of epidemic hepatitis and poliomyelitis the production of pronounced community immunity is also observed.

Community immunity is created not only as a result of epidemic outbursts, but also as planned immunization of the population. Correctly planned work in carrying out specific prophylaxis of diphtheria not only brings about a considerable decrease in incidence, but also a complete eradication of this disease which once was widespread before obligatory immunization. Due to the systematic vaccination of all the population of the USSR against smallpox over

a number of years a sound artificial immunity has been maintained preventing the occurrence of the disease as well as the existence of the smallpox virus.

THEORETICAL BASIS OF IMMUNITY

Numerous hypotheses and theories of immunity in most cases have lost their significance and are merely of historical interest.

L. Pasteur explained the reason for the immunity of the body which has endured an infectious disease as being due to an exhaustion of the medium by microbes.

A number of authors attempted to explain the principle of immunity by chemical, physicochemical and enzymatic processes occurring in the body under the influence of infectious agents.

The most well-founded theories which, to a certain extent, have not yet lost their significance is Metchnikoff's theory of the phagocytic ability of certain cells to combat pathogenic bacteria and inflammation in infectious diseases, and Ehrlich's humoral theory concerning the defensive capacity of antibodies produced by cells of the lymphoid tissue.

Between these two trends a discussion ranged for a long period which, due to the discovery of opsonin-antibodies strengthening the phagocytic reaction, led to a consolidation of the two leading schools. Tissue and humoral forms of immunity are an entity and cannot be set against one another. Tissue mechanisms (of phagocytes, inflammation, reactivity) precede the humoral defense reactions.

The further development of Metchnikoff's ideas is the study of the reticulo-endothelial system which plays a considerable part in the complex processes of tissue and humoral immunity (V. Vysokovich, L. Aschoff, A. Bogomolets, W. Taliaferro, etc.).

In 1926 V. Barykin proposed the theory that immunity is a condition brought about by the cellular and humoral systems of the body. The cellular and humoral reaction independent of its outer manifestation essentially is an entity. The origin, course and outcome of any general physiological as well as immunological reaction depends on the activity of the body as a whole. This theory is a considerable step forward in the field of studying the problems of immunity.

A. Bezredka founded the study of *local immunity*. He established that during cutaneous or intracutaneous immunization of animals against anthrax, they acquire firm insusceptibility to a virulent culture of anthrax bacilli, and do not produce specific antibodies. A. Bezredka considered anthrax to be a local infection. Arising from this point he believed that if cells sensitive to a causative agent are desensitized by vaccine they will be unable to interact with the causative agent or its toxin. According to Bezredka's theory, local immunization leads to immunity of the whole body. He also assumed that an analogous mechanism of insusceptibility is formed to intestinal, staphylococcal and streptococcal infections, since causative agents of these diseases are characterized by clearly expressed organotropy. A. Bezredka devised a method of treating pyogenic infections with staphylococcal or streptococcal (antivirus) filtrates, the use of which in his opinion blocks the sensitive skin cells and makes them resistant to pyogenic microbes and toxins. For the immunization of man to intestinal infections he suggested the method of peroral immunization. However, in later investigations it was established that local immunity does not exist as an autonomous system of defense of the body. Immunity is provided for by a complex of defense reactions of the whole body and its systems.

Of great significance in revealing the complex processes of immunity are the theories of relative dynamic constancy of the inner medium: the leading role of the central nervous system (I. Sechenov, I. Pavlov, etc.), dominants

(A. Uchtomsky), the vegetative nervous system (L. Orbeli, W. Cannon), hypophyseal-adrenal interrelationships (G. Selye), the integrating role of certain functional systems (P. Anochin), the integrating role of the hypothalamus (F. Griffits, etc.), barrier functions (L. Stern), and genetic mechanisms of immunity (G. Yerney, D. Tolmedge, F. Burnet, etc.).

Thus, immunity involves rather complex reactions which are carried out by all the systems of the macroorganism, and is regulated by the activity of the central nervous system.

REACTIONS OF IMMUNITY AND THEIR PRACTICAL IMPORTANCE

The interaction of the antigen and antibody is that of a colloid chemical reaction. It is characterized by specificity. The molecules of the antigen and antibody bind with their terminal groups (Fig. 63) as a result of which firm complexes are produced. Sometimes these compounds are reversible.

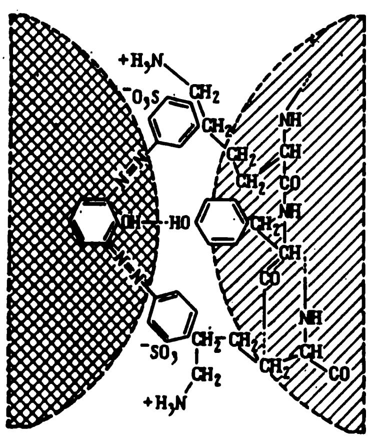


Fig. 63. Diagram of antibody and antigen binding

The interrelation of the antibodies and antigens was reduced by P. Ehrlich to ordinary chemical reactions such as the interaction of an acid (HCl) and a base (NaOH). However, this suggestion was refuted by J. Danysz who proved that toxicity of the mixture is changed depending on how the toxin is added. If an equivalent amount of toxin is immediately added to the antitoxin the mixture is nontoxic, but if it is added in separate portions then the mixture usually is toxic, since all of the antitoxin is expended on the neutralization of the first portions of toxin. Thus, the successive portions remain free and innocuous. The quantitative ratio between the antigen and antibody, according to S. Arrhenius and T. Madsen, is regulated by the law of conservation of mass, in which the product of the antigen (A) and antibody (B) concentrations in the free, unbound state are in a definite constant relation with the concentration of the compound produced $\frac{A \times B}{AB} = K$.

According to this theory, the antigen and antibody interact with one another as a weak base (ammonia) and a weak acid (boric acid). They unite incompletely due to which the ammonia and acid are always found in a free state in a considerable amount.

The interaction between the antigen and antibody, as assumed to J. Bordet, proceeds according to the colloid type of reaction. All immunological reactions between the colloid substances of antigens and antibodies have an adsorptive nature.

C. Nicolle sought to reconcile the colloidal-physical and chemical viewpoints concerning the nature of immune reactions. He regarded the mechanism

of the antibody binding with the antigen as being due to two possible variants. If the antibody and antigen are taken in optimal proportions, then the reaction between them takes place because of their relationship. When there is an excess of one of the components, then surface interaction becomes significant and the reaction acquires an adsorptive nature.

I. Ostromyslensky presumed that compounds of the toxin and antitoxin occur in two phases: adsorptive and chemical. The second phase is characterized by the production of a nontoxic, salt-like compound of the toxin with antitoxin, then this compound under the influence of oxidative processes in the blood is cleaved with the formation of antitoxic substances.

According to L. Pauling, antibodies have a bivalent structure, i.e., they contain two specific polar groups providing for the union with the antigen

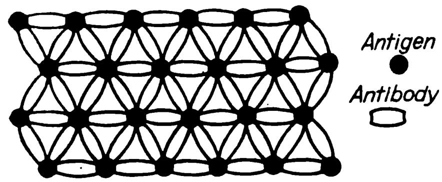


Fig. 64. Optimal proportions of antigen and antibody

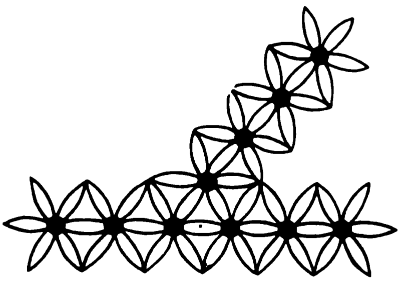


Fig. 65. Structure of the antigen-antibody complex during an excess of antibody

(Fig. 64). With an excess of antigen or antibody loose complexes are produced, and a reaction does not follow (Fig. 65). Besides bivalent (complete) there are monovalent (incomplete) antibodies (see p. 245).

One molecule of the globulin antibody, according to J. Marrack, combines with several molecules of antigen. The production of antigen-antibody aggregates is stimulated during the combination of specifically active polar groups (Fig. 66).

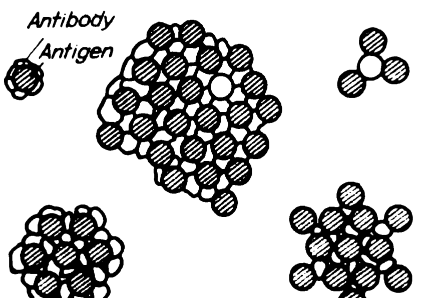


Fig. 66. Different quantitative interrelations between the antigen and antibody molecules

Chemical theories of the interaction of the antigen and antibody proposed by J. Marrack, L. Pauling, M. Heidelberger, S. Hooker and W. Boyd received the name of "lattice" theory, as the presence of polyvalent antigens and antibodies is possible, which produce upon interaction certain compounds in the form of lattice or framework composed of alternating antigens and antibodies.

After a profound investigation of antigen-antibody compounds it was shown that antibodies predominate in such compounds. Thus, for example, with the help of electron microscopy new data were revealed providing evidence that the antibodies enveloped antigens (bacteria and viruses) with a certain layer. Later it was revealed that a specific complex consisting of an antigen and antibody is capable of increasing the adsorption of nonspecific serum proteins. One of these well established kinds of reactions is the adsorption of complement by the antigen-antibody complex.

The specificity of the antibody is associated, probably, with a definite chemical group, the electronic field of which corresponds to the electronic characteristic of the antigen.

All serological reactions brought about by the interaction of the antigen and antibody occur in two phases. The first (chemical) phase is specific and is characterized by the union of the corresponding groups of antigen and antibody. The second nonspecific phase is a colloidal reaction. The serological reaction becomes defensive only when it is accompanied by the manifestation of the specific and nonspecific phases. The antigen bound with the antibody and enveloped with serum protein, in particular with complement, is capable of becoming adsorbed on the surface of various cells, including phagocytes, which provides for a perfected and complete phagocytosis.

Investigations carried out in recent years have revealed the mechanisms of immunological reactions in detail. However, many problems still remain unsolved and a whole series of propositions are only of a hypothetical nature.

ANTITOXINS AND THE REACTION OF NEUTRALIZATION OF TOXIN WITH ANTITOXIN

Antitoxins are antibodies which are capable of interacting with the corresponding toxins and neutralizing them.

As has been established some microorganisms are capable of producing exotoxins.

In the reaction of neutralization of the toxin by the antitoxin certain conditions are required—quantitative proportions, time necessary for interaction, and optimal temperature. A physicochemical reaction lies at the basis of this phenomenon. The outer manifestation of the binding of toxin with antitoxin in vitro is a flocculation, a turbidity in the test tube containing a mixture of toxin and antitoxin. Flocculation is a specific reaction, and is employed in

the manufacture of sera for the determination of the degree of activity or strength of action of antitoxic sera.

The antitoxic unit AE is accepted as the unit for measuring the strength of the antitoxic serum. AE is the dose of antitoxic serum which is capable of neutralizing a particular amount of Dlm of toxin.

The reaction between the toxin and antitoxin takes place according to the type of complex proportions calculated by special equations of adsorption isotherms. Experiments have confirmed that the free amino groups of toxins take part in rendering a toxin harmless and their binding is accompanied by the loss of toxicity.

Therapeutic action of antitoxic sera depends not only on their strength and amount of AE in 1 ml, but also on the avidity (eagerness)—rapidity, completeness and stability of the bond between the toxin and antitoxin.

Some authors consider that avidity is associated with a certain individual physicochemical condition of the colloids of sera, which does not depend on the antibody titre. Others presume that avidity depends on the quality of the serum globulins, while others associate weak avidity with the low quality of the antigen (toxin, anatoxin) employed for hyperimmunization of animals. Antigens with deformed molecules provoke the production of antibodies with a weak avidity.

Methods of obtaining and titrating sera. Antitoxic sera are obtained by hyperimmunization of animals (horses). After a complete course of immunization a large amount of antitoxin accumulates in the blood of the animals, after which partial or complete bleeding is carried out. The blood is collected in bottles where it clots, and a transparent yellowish liquid (serum) separates out above the clot.

The serum thus obtained is checked for sterility, pyrogenicity (the ability to cause an elevation of temperature in animals during parenteral injection), and the titre is established, that is, the amount of AE in 1 ml is determined.

Titration of antitoxic sera is performed with the aid of certain standard toxins on animals (guinea pigs, white mice and rabbits) by injecting the mixture of a definite dose of a standard toxin and different doses of the serum under test. Titration, for example, of the antitoxic antidiphtheric serum is performed on animals (in vivo) according to the methods of Ehrlich and Römer, and by the method of flocculation (in vitro) according to Ramon.

For titration of the antitoxin by the method of flocculation an Lf toxin is used (Limes flocculation is the amount of toxin which in 1 AE brings about the most rapid flocculation). The test tubes are placed in a water bath at 45°C (Table 11).

Table 11 shows that flocculation takes place within 10 minutes in the fifth test tube containing 0.05 ml of serum. This dose proved to be capable of neutralizing 60 Lf antigen units of toxin. Thus in 1 ml of serum there are $\frac{60}{0.05} = 1{,}200$ AE.

Table 11

Titration of Antitoxin by the Flocculation Method

Dose in ml			Time of flocculation in minutes
Serum	Toxin, containing 30 Lf in 1 ml		
0.01	2.0	60	—
0.02	2.0	60	—
0.03	2.0	60	30
0.04	2.0	60	20
0.05	2.0	60	10+
0.06	2.0	60	15
0.07	2.0	60	19
0.08	2.0	60	25
0.09	2.0	60	40
0.1	2.0	60	45

Pyrogenicity is determined by injection into rabbits of 0.1 ml of serum per 1 kg of body weight. The temperature is measured every 5, 30 and 120 minutes. If the temperature rises more than 1°C, then the serum is considered to be pyrogenic.

At present the method of determination of the toxigenicity of diphtheria (streptococcus, staphylococcus) cultures on solid nutrient media is employed for diagnostic purposes. It is based on the interaction of the antitoxic serum and toxin produced by diphtheria bacteria during growth. For this purpose, a sterile strip of filter paper soaked in antitoxic antidiphtheric serum is placed in the centre of a Petri dish and covered with a nutrient medium, after which streaks are made perpendicular to the strip of filter paper. If the culture produces exotoxin, it diffuses into the nutrient medium and the reaction of precipitation with antitoxin occurs. The precipitate thus produced can be observed as fine arrow-whiskers on both sides of the streaked culture (Fig. 114).

There are several modifications of this method, and it can be reproduced in a liquid nutrient medium.

The antitoxic sera are used in a definite amount of AE for therapeutic and prophylactic purposes in tetanus, botulism, gas gangrene, diphtheria and snake bites.

Purified sera have better therapeutic and prophylactic properties and rarely cause serum sickness.

For revealing the presence of antitoxin in the human blood serum special methods are employed by means of which the degree of intensity of antitoxic immunity is established. Thus, for example, in determining the state of susceptibility to diphtheria *a Schick reaction* is performed. The principle of this reaction is that during intracutaneous injection of a definite dose of toxin into immune persons there is no inflammatory reaction, while in susceptible

persons reddening, swelling and painfulness appear at the site of introduction of the toxin within 1-2 days. Thus, in the first case, one speaks of a negative reaction giving evidence for the presence of a definite concentration of antitoxin in the blood. In the second case, the reaction is positive, because in the blood of such people there is an insufficient amount of antitoxin for neutralizing the diphtheria toxin injected intracutaneously (the method of carrying out the Schick reaction is given on p. 422).

PRECIPITINS AND THE PRECIPITIN REACTION

Precipitins are antibodies which bring about the formation of a minute deposit (precipitate) upon interaction with a specific antigen.

The precipitin reaction is a specific interaction of the antigen (precipitinogen) and antibody (precipitin) in the presence of an electrolyte (0.85 per cent NaCl solution) with the formation of a deposit (Fig. 67) or precipitate.

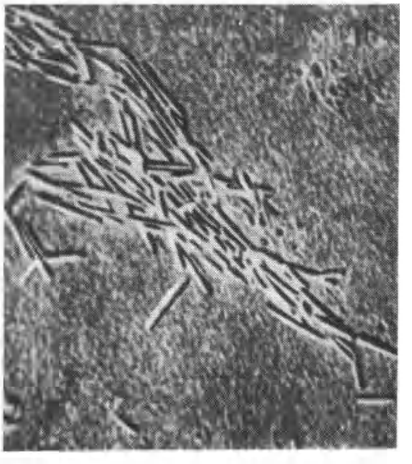


Fig. 67. Molecules of tobacco mosaic disease precipitated by antibodies

In 1897 R. Kraus proved that the transparent filtrate of a broth culture of plague bacteria became turbid when mixed with an antiplague serum with the subsequent falling-out of flakes to the bottom of the test tube. An analogous phenomenon was observed upon the interaction of cholera and typhus culture filtrates with the corresponding sera. R. Kraus named this a precipitin reaction, and the antibody producing it—precipitin.

After the injection of eel serum protein into rabbits in 1899 F. Chistovich in E. Metchnikoff's laboratory established that antibodies (precipitins) occur in their blood which, upon interaction with eel protein serum, produce a precipitate.

The precipitin reaction is most clearly obtained when the transparent filtrate (antigen solution) is layered on the transparent precipitating serum. A greyish-white ring appears (Fig. 68*a*) comparatively rapidly at the junction of the reacting components.

In mechanism this reaction is similar to the flocculation reaction and the physicochemical interrelations of highly dispersed colloids form the basis for it.

The precipitin reaction is specific and sensitive. It allows the detection of antigen (precipitinogen) in a dilution up to 1:1,000,000 and 1:10,000,000.

Precipitinogens during parenteral injection provoke the formation of specific precipitins in the body and combine with them. Proteins of animal, plant and microbe origin: blood, serum, extracts from different organs and tissues, foodstuffs of a protein nature (meat, fish, milk), filtrates of microbial cultures or affected tissues, may all be used as precipitinogens.

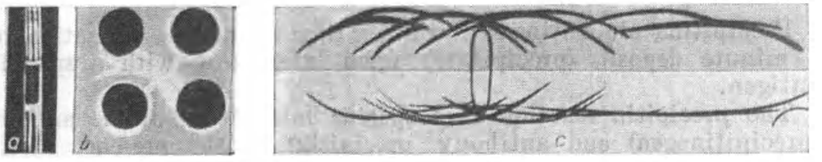


Fig. 68. Precipitin reaction

a—the precipitin reaction in a test tube; *b*—precipitation in agar; *c*—immunoelectrophoresis of normal (at the bottom) and pathological (at the top) serum

Precipitinogens of the causative agents of anthrax, plague and tularaemia are thermoresistant. Some precipitinogens withstand heat up to 120-180°C.

The precipitin reaction is used in the diagnosis of anthrax, tularaemia, etc., in the typing and studying of antigen structure of certain groups of bacteria. In forensic medicine with the aid of the precipitin reaction the origin of blood spots and sperm is determined, in sanitary examination an admixture of milk of one species of animal to another is revealed, the addition of artificial honey to natural, the falsification of meat, fish and flour goods, etc., and in biology the genetic links between related species of animals, plants and microorganisms are established.

The precipitin reaction is most widespread in the diagnosis of anthrax (Ascoli's test) for detecting the antigen of anthrax bacilli in extracts from the organs of animals, skin, wool, hair and also for the control of the manufactured goods: fur jackets, fur collars, shaving brushes and other goods. This reaction is known as *the thermoprecipitin reaction*, since the extract which undergoes investigation is preliminarily boiled, then filtered to obtain a transparent solution and layered on the precipitating anthrax serum.

The thermoprecipitin reaction (*ring precipitation*) is widely used for the diagnosis of plague and tularaemia during the investigation of extracts prepared from the internal organs (spleen, liver) of the cadavers of wild rodents.

In special laboratories there is a set of precipitating species-specific sera which are obtained by long-term immunization of ani-

mals with the corresponding antigens (precipitinogens). At the end of the immunization course blood is taken from animals (rabbits, donkeys, sheep, goats), a serum is obtained and the strength of its action is determined.

The titre of the precipitating serum is known as the maximal dilution of antigen (precipitinogen) in which a clearly expressed precipitin reaction is obtained. Whole precipitating serum is taken because the dispersion of the precipitating serum is less in comparison to that of the precipitinogen. For this reason in order to obtain optimal quantitative proportions of particles of acting components the antigen and not the serum is diluted.

In order to differentiate antibodies against different antigens, the method of the diffusion precipitin reaction with antiserum mixed with gelatine or agar is used. After the antigen solutions are layered one on the other, easily discernible precipitation zones occur within this agar, each specific pair of antigen-antibody complex having its own zone. This method was later improved. In wells made in agar in Petri dishes solutions of antigens and antibodies are poured which diffuse into the gel, and after coming in contact with one another form lines of precipitation. The merging of the ends of precipitating lines provides evidence for the similarity of antigens of comparative systems (Fig. 68*b*). At present there are several modifications.

The precipitin reaction can be carried out on paper. If the mixture of antigens is isolated by the method of paper electrophoresis and then the strips of paper are treated with immune serum, then a precipitate is produced in definite places, the localization of which determines the nature of antigen if the origin of the antibody is known, and vice versa.

The precipitin reaction became the basis of the method of immunoelectrophoresis proposed by P. Grabar and K. Williams. At first, the antigen is separated in the electric field, after which it is developed by antiserum poured in a groove running parallel to the line along which the antigens moved during electrophoresis. Each antigen gives an individual band with the antibody. From the number and arrangement of the bands one can determine the presence of these or other antigens in the solution under test. With the help of immunoelectrophoresis antigen fractions which were unknown earlier have been revealed in various complexes. It allows to detect pathological deviations in the sera of patients (Fig. 68*c*). The precipitin reaction procedure is described in practical manuals.

AGGLUTININS AND THE AGGLUTINATION REACTION

Agglutinins are antibodies capable of clumping the corresponding microbes by producing visible conglomerates.

The addition of the corresponding immune sera to a suspension

of microbes provokes clumping of microbial cells in the form of flakes or granules (Fig. 69). This phenomenon is known as agglutination. The agglutination reaction takes place on mixing erythrocytes, yeasts and other cells with the corresponding immune sera.

It was described by A. Charrin, G. Roger (1889), V. Isaiev and V. Ivanov (1894) and was investigated in detail by M. Gruber and H. Durham in 1896. In the same year F. Widal reported that the serum of typhoid patients was capable of specifically clumping (agglutinating) typhoid bacteria. Later it was established that in a whole series of infectious diseases antibodies (agglutinins) are produced in the blood of patients, which are capable of clumping the corresponding causative agents of infectious diseases.

Fig. 69. The agglutination reaction (formation of "spiders")

The agglutination reaction, like the flocculation and precipitin reactions, is under the control of the physicochemical conformities of the interrelations of colloidal systems. The antibody (agglutinin) and antigen (agglutinogen) take part in the agglutination reaction. Their interaction takes place in definite quantitative proportions, and in the presence of an electrolyte (0.85 per cent NaCl solution). In mechanism and outer manifestation the agglutination reaction is similar to the precipitin reaction. Both reactions are accompanied by the production of visible precipitates of antigen with the difference that in the agglutination reaction microbial bodies serve as the antigen, in the precipitin reaction the antigen is the product of the breakdown of microbial bodies, very minute particles of dissolved antigens requiring a large amount of antibodies for complete interaction.

The agglutination reaction is characterized by specificity, but group agglutination can be found, that is, the clumping of closely related microbes though in weaker serum dilutions.

The antigenic structure of bacteria is quite varied. In one and the same bacterial strain there may be group, species, and type antigens. Similar bacteria are composed of various antigenic particles, and during immunization of animals the corresponding agglutinins are produced in the blood. This can be represented in the table shown on the next page.

As may be seen from this table, the serum received against microbe A agglutinates microbe A readily, since agglutinins $a_1b_1c_1$ complete-

Species of bacteria	A	B	C
Antigens (agglutinogens)	abc	bcd	cde
Antibodies (agglutinins)	$a_1b_1c_1$	$b_1c_1d_1$	$c_1d_1e_1$

ly correspond to the agglutinogens abc. This serum agglutinates microbe B (to a lesser degree) due to the homologous b_1c_1-agglutinins and bc-agglutinogens, and also microbe C (to an even lesser extent) due to the common character of c_1-agglutinin and c-agglutinogen. These interrelations are found between the serum against microbe B and microbes B, C and A, etc.

Thus, upon immunization of the animal with one species of microbe agglutinins may occur not only to this species, but to other related bacterial species which have general group antigens.

For revealing specific agglutinins in sera of animals immunized by a complex of antigens of the bacterial cell *the method of adsorption of agglutinins* is employed (Castellani's exhaustion reaction). By adding certain species of bacteria to the serum of an immunized animal, in which there are several agglutinins, those which clump only organisms of this species are removed, after which the serum freed from these agglutinins is checked for the presence of other agglutinins by adding other species of bacteria.

For example, an agglutinating serum causes clumping of A and B bacteria. It is necessary to establish whether these agglutinins are the result of the antigenic action of microbes A and B or whether only one of them is a group antigen. For this purpose microbe A which will be agglutinated by the corresponding antibodies is added to the agglutinating serum. Then this serum is treated with the A microbe, it is then filtered, and with the filtrate of this serum the reaction of agglutination with microbe B is carried out. If the formation of agglutinins was brought about by the action of antigen A then the agglutination reaction with microbe B does not occur. If this experiment is carried out at first with microbe B then it will remove its own agglutinin, but after depletion the agglutinins to microbe A remain. Consequently, in this case the agglutinins to microbe B are group agglutinins. If agglutination took place after the depletion of the serum by either the A or B microbe, then it should be considered that the agglutinins were produced under the influence of both antigens.

The antigenic structure of bacteria can be studied by the method of agglutinin adsorption, which is used for preparing agglutinating and therapeutic sera, vaccines and diagnosticums. Agglutinating sera obtained by the method of agglutinin adsorption are known as monoreceptor. They allow a more accurate determination of the species and type specificity of the causative agent of salmonellosis and dysentery.

In motile microbes there are flagellar (H) and somatic (O) antigens. During immunization of animals with motile bacteria H-agglutinins and O-agglutinins are correspondingly produced. Flagellar agglutinins cause a more rapid clumping of microbes in the form of loose flakes, while somatic agglutinins produce compara-

tively slowly conglomerates of bacteria in the form of fine granules (Fig. 70). H-agglutination is otherwise known as large flaky and O-agglutination as fine granular agglutination.

Bacteria containing the Vi-antigen are only weakly or even not agglutinated by O-sera, but agglutinate well with Vi-sera. This shows that O- and Vi-antigens as well as O- and Vi-antibodies have a different structure.

A series of observations and investigations have established that certain microbes of the group of normal microflora of the intestine under the influence of a causative agent acquire the property to agglutinate with patient's sera.

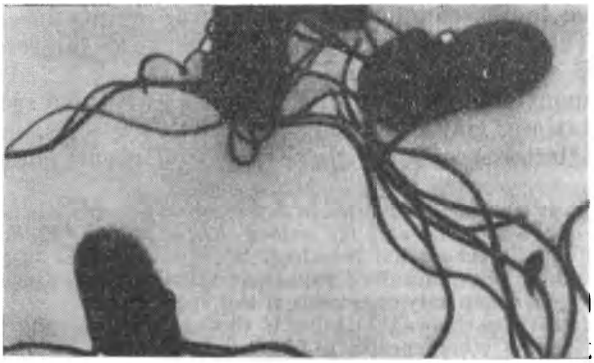


Fig. 70. Agglutination of typhoid bacteria by flagellar agglutinins

Thus, for example, during dysentery the colibacillus is sometimes agglutinated by the dysentery serum. This property of many microbes (which are not causative agents) to be agglutinated with a patient's serum from which they were isolated is known as *paragglutination*, and the cause of its occurrence, as *paraimmunity*.

The phenomenon of paragglutination occurs due to the variation in microbes, first of all, in antigen structure. Under the influence of microbes more active antigenically, comembers of microbial associations acquire general antigenic characters. The mechanism of this process, as is assumed, consists in that the desoxyribonucleic acid of one species of microbe may provide for the transformation of another species comember of a microbial association or parasite coenosis. Then this other species of microbe acquires the property of the former species (see "Genetics of Microorganisms").

The reaction of agglutination may take place as a result of the action of nonspecific factors (without the presence of an agglutinating serum), the main colloidal solutions of dyes and acids. Such nonspecific reactions may also take place in the presence of an isotonic solution alone in microbes which were exposed to considerable changes as a result of long storage, and also in R-forms of bacteria.

The extent of manifestation of the specific agglutination reaction depends on the salt concentration (electrolyte), serum concentration, density of bacterial suspension, pH, influence of temperature, shaking and mixing, etc.

Negative zones of agglutination which depend on the concentration of agglutinins are of special significance in serological reactions. In very high and also in low serum concentrations the agglutination reaction does not take place. These peculiarities are characteristic of all immunity reactions and should be taken into consideration in practical work during diagnosis of infectious diseases.

The agglutination reaction is widely employed in the practice of serological diagnosis of enteric fever, paratyphoids A and B (*Widal's reaction*), brucellosis (*Wright's reaction*), typhus fever (*reaction with Rickettsia prowazeki*), tularaemia, leptospirosis and other diseases, in which with the help of known microbes (diagnosticums) the corresponding agglutinins are determined in patients' sera.

For determining Vi-antibodies in carriers of enteric fever salmonellae Vi-agglutination has had wide application in laboratory diagnosis.

The agglutination reaction is used for the identification of isolated microbes in patients and sick animals with the application of previously known agglutinating sera.

Besides direct agglutination, in the diagnosis of infectious diseases *indirect agglutination* is employed. The most effective is indirect haemagglutination. The essence of this reaction is that the antigen is adsorbed on the surface of sheep erythrocytes, then the agglutination reaction is carried out with these erythrocytes and patients' sera. In particular, this reaction is employed for the diagnosis of typhus fever and tuberculosis (Middlebrook-Dubos test).

Indirect agglutination (Boyden's reaction) is used for detecting antibodies produced against antigens which do not have a corpuscular structure (protein complexes). For this purpose erythrocytes are preliminarily treated with a weak solution (1:20,000-1:80,000) of tannic acid for 10 minutes at 37°C. After this the antigen is added to the suspension of erythrocytes and adsorption occurs within a few minutes at room temperature. This reaction is quite sensitive and allows the detection of antibodies in greater dilutions.

To obtain a quick response accelerated agglutination reactions are used as tentative methods in some cases: *Nobel's reaction* for detecting typhus and enteric fever, *Huddleson's reaction* for brucellosis, *Minkevich's reaction* for typhus fever and tularaemia and *the agglutination reaction with luminescent sera* for revealing causative agents of intestinal infections, anthrax, etc.

In surgical practice of blood transfusion the reaction of isohaemagglutination has had wide application with the help of which blood groups may be determined. For this purpose it is necessary to have two haemolytic sera (β and γ) obtained from people with A and B blood groups. One or two drops of each of these sera are put separately on a slide or china dish, and one small drop of the blood under test is added. The blood and serum are carefully mixed and, according to the reaction of isohaemagglutination, the blood group is established (Fig. 71).

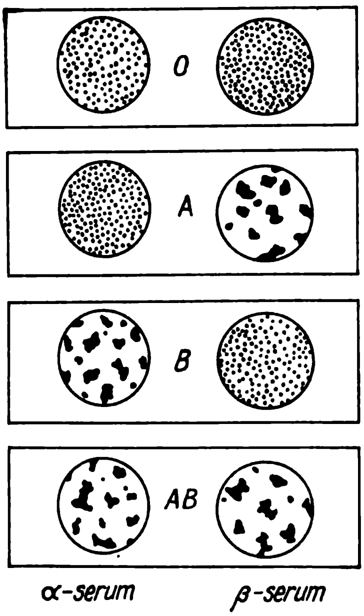


Fig. 71. The isohaemagglutination reaction

To obtain agglutinating animal sera (rabbit, etc.), the animals are immunized with a suspension of freshly isolated bacteria of a certain species or type according to a certain schedule, taking into account the dose and the intervals between vaccinations. At the end of immunization blood is taken from the animals and the serum obtained is inactivated, conserved and titrated. The titre of the agglutinating serum is known as the smallest amount or the greatest dilution which causes a clearly marked agglutination reaction. On the labels of ampoules of manufactured sera the titres are written as fractions indicating the maximal dilution (1:3,200, 1:6,400, 1:12,800, 1:25,600, etc.) at which they cause agglutination of the corresponding antigen (agglutinogen). Agglutinating sera are produced as nonadsorbing and adsorbing, multivalent, species and type specific.

LYSINS AND THE LYSIS REACTION

Lysins are specific antibodies which cause the dissolution of bacteria, plant and animal cells.

Under the influence of antibodies and a substance contained in normal serum, complement, the dissolution of microbial cells (bacteriolysis) takes place, or bactericidal action accompanied by destruction of microbes without any noticeable morphological changes occurs.

In 1884 V. Gromann established the bactericidal action of normal serum on the microbes of anthrax. V. Isaiev and R. Pfeiffer revealed antibodies (bacteriolysins) dissolving bacteria in the blood of immune animals. The Isaiev-Pfeiffer phenomenon may be reproduced in guinea pigs, actively or passively immunized against cholera. When a culture of cholera vibrios is injected into the peritoneal cavity of an immunized guinea pig, they lose their motility fairly rapidly, swell, become spherical, then granular and then finally completely disappear and dissolve (Fig. 72). The same phenomenon is observed during simultaneous injection of live cholera vibrios and anticholera serum into a guinea pig.

E. Metchnikoff and J. Bordet established that bacteriolysis may be observed outside the body by adding fresh immune serum to a bacterial suspension. In

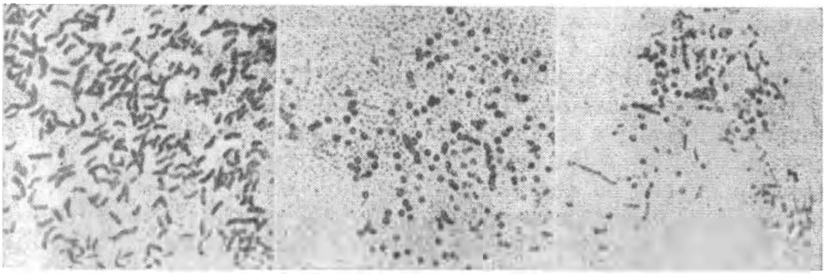


Fig. 72. Subsequent stages in the lysis of cholera vibrios

later investigations it was established that bacteriolysis depends not only on the antibody which appears under the influence of immunization, but also on the thermolabile substance (*complement*) found in all kinds of fresh serum, and which is disintegrated by heating at 56°C for half an hour.

Thus, bacterial lysis takes place with the participation of two components: a specific antibody contained in the immune serum and a nonspecific substance of any normal or immune serum—complement.

The antibody which together with complement causes bacterial lysis was named sensitizer by J. Bordet and—ambocaptor by P. Ehrlich. It is usually called *lysin*—bacteriolysin, haemolysin, etc.

Lysins are capable of dissolving bacteria, treponema, leptospira and also of profoundly impairing the structure of erythrocytes, leucocytes and other cells. They are characterized by all the main properties of antibodies with the difference that the antigen is acted upon in the presence of complement.

Complement is a protein consisting of a mixture of globulins and mucoprotein, the origin being the same as that of other globulins in the body. E. Ecker assumed that complement is similar to certain hydrolytic enzymes and contains a sulphohydryl group.

At present it has been established that complement is composed of several fractions C′1, C′2, C′3, C′4, C′5, C′6. Fraction C′1 is an euglobulin, C′2 and C′4 pertain to mucoeuglobulins, while fraction C′2 is thermolabile, and C′4 is relatively thermoresistant. Fraction C′3 under the influence of zymase is inactivated at 37°C and deprives the serum of its complement-fixing properties.

The complement is quite sensitive to heat, and disintegrates at 56°C in half an hour, and also during long-term storage, prolonged shaking, under the influence of ultraviolet rays and chemical substances. The complement is easily adsorbed on the surface of various substances—aluminum hydroxide, kaolin, animal charcoal, bacterial suspensions, erythrocytes, etc.

Due to the lability of complement methods have been devised for its storage. Preservation of complement (usually guinea pig

serum) is carried out by adding either sodium chloride of a 10 per cent concentration, 4 per cent boric acid, or 5 per cent sodium sulphate, and also by drying at a low temperature in a vacuum.

Haemolysis reaction. During immunization of rabbits with a suspension of sheep erythrocytes antibodies accumulate in the blood capable of altering the erythrocytes, as a result of which the adsorption bond between the haemoglobin and stroma is impaired. The haemoglobin easily passes out of the erythrocytes into the surrounding fluid, and is coloured pink. Heating the immune serum at 56°C for 30 minutes is accompanied by the loss of haemolytic properties due to the disintegration of complement. The addition of fresh animal serum, even a nonimmune serum, restores the haemolytic properties of the immune serum. If haemolytic serum (antibody), sheep erythrocytes (antigen) and complement are placed in a test tube in definite quantitative proportions, then in a few minutes a change will take place in the mixture. It changes from turbid red to pink (lacquered) as a result of haemolysis (haemoglobin goes out of the stroma of erythrocytes).

The reaction of haemolysis has a strictly marked specificity. It is used as a component part for carrying out the complement-fixation reaction.

THE COMPLEMENT-FIXATION REACTION

The specific interaction of the antibody and antigen is accompanied by the adsorption of complement. As the process of adsorption of complement by the antigen-antibody complex could not be observed, in 1901 J. Bordet and O. Gengou introduced into this reaction as an indicator a second system (*haemolytic*) composed of a suspension of erythrocytes and the corresponding haemolytic serum, with the help of which the fixation of complement was revealed.

In the complement-fixation reaction two systems are involved: antigen+antibody+complement (*first system*), and suspension of erythrocytes+haemolytic serum (*second system*). Both systems are placed separately in a thermostat for half an hour. Then they are mixed, poured into one test tube, and the mixture is placed again in a thermostat for 30-60 minutes. If the complement-fixation reaction proves to be positive, that is, if complement is bound to the antigen-antibody complex of the first system, then the second system (erythrocytes+haemolytic serum) does not change as no complement is left for it (Fig. 73*a*). Within a day the erythrocytes settle to the bottom, and the supernatant liquid is transparent and colourless. During a negative reaction the complement will not combine with the complex of the first system (Fig. 73b), while the liquid becomes pink (lacquered) without any precipitate of erythrocytes.

The complement-fixation reaction has a high specificity and a marked sensitivity.

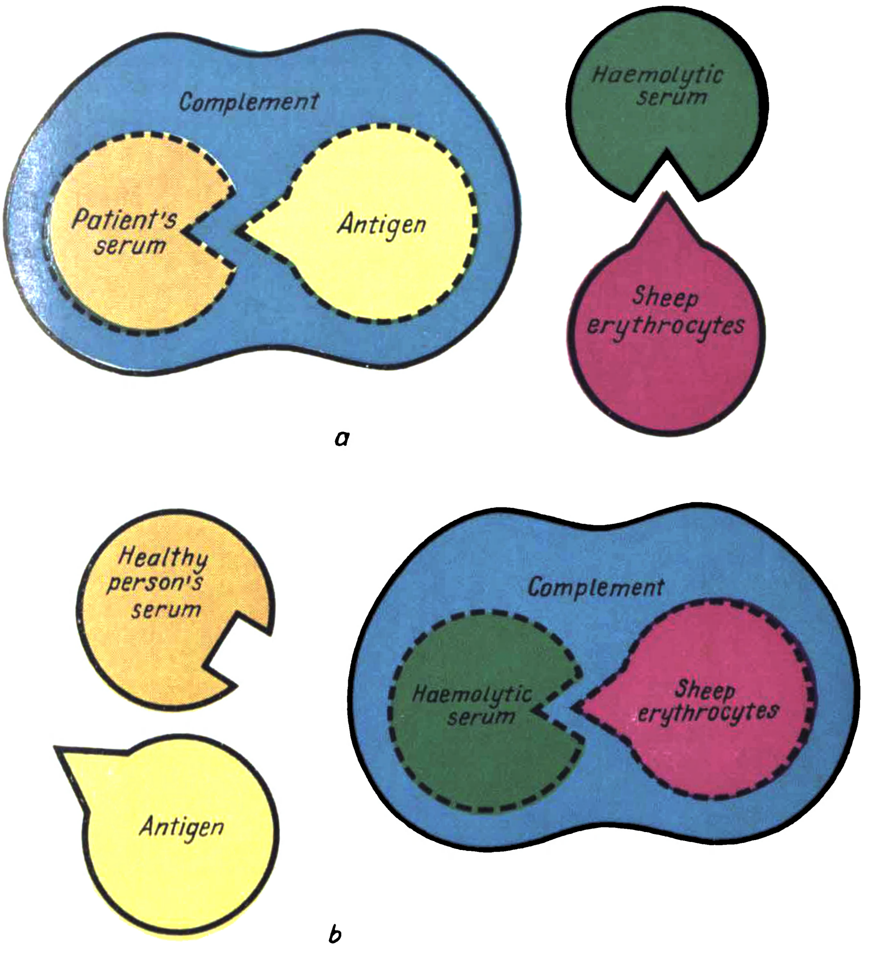


Fig. 73. Diagram of the complement-fixation reaction

a—positive; *b*—negative

According to the mechanism of action this reaction is the most complex in comparison to reactions of agglutination and precipitation and proceeds in two phases. In the first phase precipitation occurs between the antigen and antibody (mutual adsorption), and in the second, fixation of the complement by the antibody-antigen complex takes place.

Complement participates in all immunological reactions, while in some reactions the presence of complement is obligatory (lysis, complement-fixation), in others it is nonobligatory (neutralization of toxin by antitoxin, precipitation, agglutination and opsonization).

The complement-fixation reaction is used in the diagnosis of glanders, syphilis (Wassermann reaction), etc. In recent years it has been used successfully in discerning typhus fever, Q fever and other rickettsioses and many viral diseases. Modifications of the complement-fixation reaction have been devised for the purpose of determining antibodies as well as antigens in the blood of patients.

Blocking antibodies. Interrelations between protective (defensive) antigens and the corresponding antibodies are of a completely different nature. During this interaction no typical immunological reactions are observed (neutralization, precipitation and complement-fixation). It has been suggested that protective antigens provoke the formation of incomplete or blocking antibodies capable of rendering harmless the agressins of anthrax bacilli, capsular proteins of the causative agents of plague, tularaemia and of other bacteria.

Incomplete (monovalent) or blocking antibodies are fixed by the antigens, but do not cause their agglomeration. In contrast to ordinary (complete) antibodies they proved to be more stable to heat, pressure, and chemicals, and quite easily penetrate through the placenta. They include rhesus-agglutinins*, nonprecipitating thermolabile antibodies and reagins of allergic patients, and of patients with systemic lupus, infectious polyarthritis, and collagenosis.

Of most interest are agglutinins against the rhesus-antigens of erythrocytes of children suffering from haemolytic disease which is the result of the presence of a rhesus-factor in the erythrocytes inherited from the father. After penetrating into the blood of the mother the rhesus-factor provokes the production of rhesus-agglutinins which later enter the blood of the foetus through the placenta and cause agglutination of erythrocytes. Haemolytic disease is due to the incompatibility of the rhesus-factor in the blood of the mother and the foetus.

The rhesus-factor is capable of causing the production of two types of agglutinins: (1) complete (bivalent) agglutinins which in

*In the erythrocytes of monkeys of the species Macaca rhesus an antigen was discovered which is found in human erythrocytes. Hence the name rhesus-factor.

a saline and colloidal medium may cause the agglutination reaction of erythrocytes containing a particular antigen, and (2) incomplete (monovalent) agglutinins inhibiting agglutination, which do not cause the agglutination reaction in a saline solution.

For detecting incomplete antibodies special methods are employed. The Coombs' test is used, in particular to detect incomplete agglutinins in rhesus-negative mothers. To determine the fixation of agglutinins by the patients' erythrocytes, antiglobulin serum is added, which, in a saline solution, is capable of causing marked agglutination of erythrocytes sensitized by incomplete agglutinins. A molecule of antiglobulin binds two molecules of incomplete agglutinins fixed to two different erythrocytes, due to which the agglutination reaction takes place (Fig. 74).

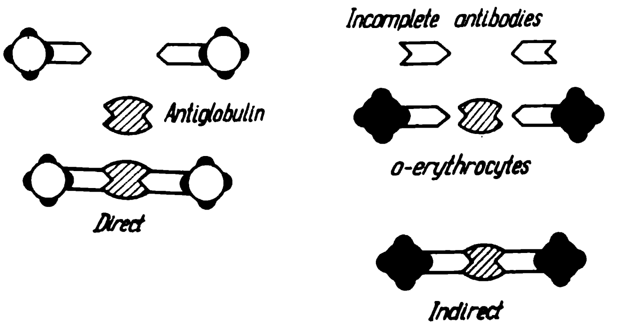


Fig. 74. Coombs' test

OPSONINS AND THE OPSONOCYTOPHAGIC REACTION

Opsonins are antibodies of normal and immune sera which alter the microorganisms and prepare them for a more intensive phagocytosis. Under the influence of opsonins a change takes place in the properties of the surface of microbial and other antigens, in particular, in their electrical potential due to which they are easily exposed to phagocytosis.

E. Metchnikoff and J. Bordet noticed that the phagocytic activity of leucocytes reveals itself better in the presence of immune serum than in normal serum. They explained this phenomenon by the presence of specific substances (stimulins) in immune sera.

A. Wright, S. Douglas and I. Savchenko, et al, confirmed the presence in immune sera of substances stimulating phagocytosis. These substances were named opsonins (Gr. *opson*—food) by A. Wright. Opsonins upon heating at 56°C for 30 minutes are inactivated, as they contain complement.

F. Neufeld and V. Rimpau revealed thermostable substances in immune sera which do not contain complement, and named them tropins (*bacteriotropins*).

Opsonins and bacteriotropins bring about specific sensitization and increased sensitivity of bacteria to phagocytosis.

F. Neufeld, I. Savchenko, et al, established that the thermolabile fraction does not differ in any way from complement. The thermostable fraction bearing the function of antibodies reinforcing phagocytosis has the main opsonizing action. Complement strengthens the action of bacteriotropins.

Thus, the mechanism of phagocytosis is similar to that of bacteriolysis. The specific sensitization of bacteria by antibodies is common to both processes and prepares them for lysis outside the cell (bacteriolysis) or inside the cell (phagocytosis).

The degree of activity of opsonins is accepted as the *opsonic index* which is the ratio of the phagocytic index of the immune serum to the phagocytic index of normal serum. The phagocytic index is determined by dividing the number of microbes absorbed by 100 phagocytes by the number of phagocytes. The opsonic-phagocytic index is estimated by means of a special formula which gives a more accurate result. The opsonic index should be more than 1.

$$\text{Opsonic index} - \frac{\text{phagocytic index of immune serum}}{\text{phagocytic index of normal serum}} > 1$$

In the practice of laboratory diagnosis of some infectious diseases (gonorrhoea, brucellosis, etc.) not the opsonic index, but the opsonocytophagic test is used which consists in counting the phagocytized bacteria in 25 segmented neutrophils. The results of the reaction may be different (Table 12). They vary from 0 to 75. In a healthy man, the index of phagocytic activity is 0-5, while values of 10-24 are considered to be weakly positive, and values of 25-49, clearly marked, and those of 50-75, distinctly positive. In hospitals for patients with infectious diseases various modifications of the opsonocytophagic test are used.

Table 12

Calculation of the Opsonocytophagic Test During Brucellosis

The number of brucellae phagocytized by one neutrophil	Evaluation of phagocytosis	Number of leucocytes (neutrophils)	Estimation of the numerical unit
0	0	3	$3 \times 0 = 0$
1—20	+(1)	4	$4 \times 1 = 4$
21—40	++(2)	6	$6 \times 2 = 12$
41 and more	+++(3)	12	$12 \times 3 = 36$
		25	Index 52

Thus, all serological reactions may be subdivided into two groups, simple and complex. Simple reactions may be direct (reactions of neutralization, precipitation and agglutination) or indirect (reaction of inhibition of haemagglutination). The complement-fixation reaction may be included in complex serological reactions.

Two components take part in simple serological reactions, the antigen and antibody, and in a simple indirect reaction three components are necessary. For example, the antigen, antibody, and erythrocytes, on the surface of which the antigen is adsorbed, take part in the haemagglutination inhibition reaction.

Complex reactions include two systems: antigen, antibody and complement—the first system, and the haemolytic serum and sheep erythrocytes—the second system.

Serological reactions between viruses and the corresponding antibodies are described in a special section of this textbook.

ALLERGY AND ANAPHYLAXIS

C. Pirquet gave the name allergy (Gr. *allos*—other, *ergon*—action) to the altered reactivity of the body under the influence of the action of pathogenic microbes, toxins, medicines and other substances.

Allergy is an altered reactivity of the body which manifests itself in the disturbance of the usual course of general or local reactions, often during repeated entrance into the body of substances known as allergens. These reactions may be increased in comparison to the standard, that is, strengthened and speeded up (*hyperergia*), or lowered, that is, weakened and slowed down (*hypoergia*), or these reactions may be completely absent (*anergia*), for example, during absolute immunity.

ANAPHYLAXIS

Allergy is manifested in several forms of altered reactivity of the body. One of these is anaphylaxis (Gr. *ana*—against, *filaxis*—defense), the state of increased sensitivity of the body caused by a repeated injection of foreign proteins (sera, antibiotics, etc.).

In 1839 F. Magendie obtained an increased sensitivity in rabbits which perished from the third injection of egg white, which had been completely harmless during the first injection. Analogous phenomena were described in 1894 by S. Flexner. In his experiments animals perished from a second injection of dog serum. In 1888 C. Richet and J. Hericourt noticed that during repeated injections of eel serum into dogs the latter did not acquire resistance to it but, on the contrary, became quite sensitive and perished.

In 1902 C. Richet and P. Portier established an increased sensitivity in dogs to repeated (within 11-12 days after the first injection) administrations of extracts from the tentacles of *Actinia*. This state of increased sensitivity to repeated injections of protein was named anaphylaxis by C. Richet.

In 1902 T. Smith easily reproduced anaphylactic shock in guinea pigs by the injection of horse serum.

Further investigations of the mechanisms of altered reactivity of the body were carried out by G. Sakharov, A. Bezredka, M. Rosenau, and others.

The first dose of the antigen (protein) causing increased sensitivity is known as the *sensitizing* dose (Fr. sensibiliser—to make sensitive). The second dose, from the injection of which *anaphylactic shock* develops, is known as the *rective* dose. The sensitizing dose is injected into animals subcutaneously, intraperitoneally, intravenously, or by the intracardiac route; and the reacting dose is injected intravenously or by the intracardiac route and in a larger amount than the sensitizing dose.

The state of increased sensitivity in animals does not develop immediately, but after a certain incubation period (8-21 days).

The clinical picture of anaphylactic shock is various in different species of animals. The best example of anaphylactic shock can be reproduced in guinea pigs. If a guinea pig is preliminarily sensitized by a subcutaneous injection of 0.01 ml of horse serum, and then within 8-21 days 0.1-0.5 ml of the same serum is injected directly into the heart, then anaphylactic shock develops quite rapidly. Within 1-2 minutes the guinea pig becomes restless, the fur stands on end, the guinea pig begins to rub its nose with its paws, involuntary defaecation and urination, sneezing, acute dyspnoea, tonic and clonic spasms are observed and breathing becomes slower and difficult. Within 5-10 minutes death of the animal occurs from asphyxia, with a fall in temperature, decrease of complement and impairment of blood clotting. Autopsy reveals emphysema of the lungs due to a spasm of the bronchial muscles, inability of the blood to clot, and hyperaemia and haemorrhages in the mucous membrane of the stomach, intestine and other organs.

In dogs anaphylaxis is accompanied by a spasm of the hepatic veins causing the phenomena of stasis in the liver and an insufficient blood supply to the heart. The animal dies from collapse. In rabbits anaphylaxis is characterized by a spasm of the end arteries of the pulmonary circulation, a blockade of the pulmonary circulation, a drop in blood pressure in the systemic circulation, slowing down of cardiac activity and a sharp dilation of the right ventricle. Death results from respiratory arrest and drop in the blood pressure.

In man all three types of anaphylaxis are observed. However, the type pertinent to guinea pigs occurs predominantly. Spasm of smooth muscles is an important factor in the mechanism of the development of anaphylaxis.

Anaphylactic shock in humans arises from repeated injections of immune sera during treatment of patients with various infectious diseases (diphtheria, tetanus, anthrax, gas gangrene), and of antibiotics (penicillin, etc.). It is characterized by a series of symptoms: dyspnoea, quick pulse, cold limbs, temperature rise, spasms, oede-

mas, pains in the joints, body rash, and affection of the central nervous system, sympathetic and parasympathetic systems, etc. In some cases anaphylactic shock terminates in death.

In the mechanism of anaphylaxis a definite role is played by the reaction of the antigen and antibody in tissues. The union of the antigen with the antibody fixed in the cells stimulates the cells, and a pathological process develops in the tissues, as a result of which the smooth muscles contract.

Allergic processes in general and anaphylaxis in particular are accompanied by a disturbance in tissue respiration and constitute quite a complex process developing in the sensitized body. The phenomena of allergy and anaphylaxis are inherent to comparatively highly organized bodies, capable of reactivity. They were preceded by simple forms of parasitism, and septic and toxic infections.

Local manifestation of anaphylaxis. Repeated subcutaneous injections of horse serum to rabbits at six day intervals cause an infiltration and necrosis of tissues (Arthus phenomenon) after the 5th-7th injection. Local anaphylaxis develops after the injection of other antigens (bacteria, toxins, antibiotics, etc.).

Anaphylaxis may be reproduced in sensitized animals and in separate isolated organs (Schultz-Dale reaction), containing smooth muscles (uterus, segments of the intestine, etc.).

Passive anaphylaxis. An increased sensitivity may be reproduced in normal guinea pigs passively, that is, by injecting immune serum of sensitized animals. The state of sensitization does not appear immediately, but within 24 hours after subcutaneous injection, within 12 hours after intraperitoneal injection and within 4 hours after intravenous injection. Increased sensitivity is retained in guinea pigs from 3-4 weeks to 2 months.

Desensitization (antianaphylaxis). If the reacting dose does not provoke anaphylactic shock then the animal loses its increased sensitivity to this antigen, desensitizes on the 2nd-3rd week, and then becomes sensitive again. sometimes to an even greater extent. Desensitization ensues also after anaphylactic shock. A. Bezredka suggested a specific, simple and quite an effective method of desensitization by a fractional injection of the antigen (serum).

This method is widely used in practical medicine. For desensitization of the body during serotherapy only 0.5-1 ml of the serum is injected at first, and within 1-2 hours the full dose is given. To avoid anaphylactic shock, the method of fractional injection of the serum is employed at present. At first 0.1 ml of serum is injected subcutaneously, followed by another 0.2 ml within 30 minutes, and within 1 hour the rest of the dose prescribed for the patient is injected intramuscularly.

Anaphylactic shock may be averted by nonspecific substances, e.g., the injection of a reacting dose of serum under ether anaesthesia, or by the effect of chloralhydrate and alcohol. Dimedrol, diproz-

ine (pipolphen), atropine, ether, chloroform, urethan, novocain, bile acid salts, saponin, hirudin, sodium hyposulphite, calcium chloride, etc., have desensitizing properties.

Anaphylaxis like immune reactions is characterized by specificity. To arouse it, a preliminary sensitization with a definite antigen is necessary. Substances causing sensitization have the properties of a complete antigen. Anaphylactic shock may be caused by incomplete antigens (corresponding to haptenes).

Mechanism of anaphylaxis. Many regard anaphylaxis as the consequence of the interaction of the antigen and antibody accompanied by the formation of toxic substances. According to E. Friedberger, preliminary sensitization of the body leads to the production of antibody (anaphylactin), and the subsequent injection of the reacting dose of the antigen (anaphylactogen) causes the production of a complex (anaphylactin and anaphylactogen). Under the influence of complement this complex is changed and a toxic substance is formed known as anaphylatoxin. However, such an explanation cannot completely reveal the principle of the complex mechanism of anaphylaxis.

J. Bordet considered that anaphylactic shock occurs as a result of the production of serotoxin. He established that if the serum is treated with an agar suspension it becomes slightly opalescent and causes shock manifested by symptoms similar to those in anaphylaxis.

Many investigators have noticed that during anaphylactic shock a change occurs in the colloidal state of serum proteins in the blood of animals. Roughly granular particles of protein are formed which cause embolia or bring about the stimulation of nerve endings in the intima of the vessels.

Further investigations revealed that anaphylactic shock may be caused by the action of histamine which calls forth bronchial spasms in guinea pigs and a drop in blood pressure in dogs, etc.

Histamine is produced by decarboxylation of the amino acid histidine. Although its importance in metabolism is not clear histamine plays an important part in all allergic conditions.

Serotonin (5-hydroxytryptamine) which is a derivative of triptophan has an effect on the vascular system. Heparin (polysaccharide) has an anticlotting action on the blood.

SERUM SICKNESS

Serum sickness develops within 8-12 days after a single primary introduction usually of large doses of serum (from 10 ml and more). Bezredka's method does not avert serum sickness. In some cases in sensitized people serum sickness ensues quite rapidly after the injection of serum, and then it resembles anaphylactic shock. Serum sickness

is characterized by the appearance of a rash resembling urticaria and is accompanied by a severe itch, elevated temperature, oedema, pains in the joints, swelling of the lymph nodes, disturbances of the cardiovascular activity, and a change in the white blood count (first leucocytosis, then leucopoenia and relative lymphocytosis). The patient recovers within a few days.

The mechanism of serum sickness as that of anaphylactic shock is based on the interaction of the antigen and antibody. According to C. Pirquet and B. Schick, serum sickness occurs as a result of the interaction of the antibodies produced during the incubation period with an antigen which had been retained in the body.

Serum sickness is prevented by the use of matured therapeutic sera, sera preliminarily heated at 56°C for 0.5-1 hour, or sera purified from ballast protein fractions. The above mentioned methods of treating sera lower their toxic action. Treatment of serum sickness is carried out with dimedrol, diprasin and other antihistamine preparations.

OTHER ALLERGIC DISEASES

Besides sera which call forth anaphylaxis and serum sickness various substances may serve as allergens. These agents are subdivided into: *household* (dust from feather quilts, pillows, skin epidermis, dandruff from dogs, cats, horses, etc.), *industrial* (library dust, dust from wool, cotton, minute hairs, certain dyes, soaps, varnishes, wood pulp, explosive and synthetic substances, etc.), *plant* (plant pollen during flowering of alder, hasel-nut, elm, poplar, and birch trees, meadow grass, garden and potted plants of timothy grass, meadow grass, tulips, primroses, dahlias, roses, etc.), *food* (meat, fish, eggs, honey, alpine strawberries, strawberries, shellfish, citrus fruits, coffee, chocolate, etc.), *medicinal*, *infectious*, etc.

The state of allergy in some cases is caused by the action of overheating, cooling and strong wind. Thus, for example, under the influence of an intensive tan urticaria appears, while cooling in the winter period leads to spells of asthma, rhinitis, and other more severe manifestation of the allergic reaction.

Dermatitis on the hands of bakers when employing additives to yeast, and rashes on the skin of women from the effect of a compound which is used for hairdressing are included in the reactions of increased sensitivity to chemical substances.

In recent years autoimmune diseases have attracted attention including agammaglobulinaemia (see p. 218), Hashimoto's disease involving a stable enlargement of the thyroid gland with manifestations of hypothyroidism. During Hashimoto's disease the tissue of the thyroid gland is packed with lymphocytes and plasmatic cells which are not normally found. At the site of injury spe-

cific antigens are excreted from the thyroid gland and leucocytes and other mesenchymal cells penetrate into the gland. During rheumatoid arthritis the rheumatoid factor (complex molecule of globulin) reacts with the impaired human gamma-globulin. Under the influence of traumas, overstrain, the action of cold, heat and other factors the antigen is produced which brings about its interaction with the cells of the joints. Autoimmune diseases probably include acute rheumatic fever (see p.229) and acquired haemolytic anaemia, during which incomplete antibodies capable of blocking erythrocytes not only in sick but also in healthy people are produced in the blood. In the pathogenesis of this disease the main reaction comprises the combination of antibodies with erythrocytes. The cause of systemic lupus erythematosus which occurs due to the production of antibodies against the DNA of the nucleus also is an autoimmune mechanism. The synthesis of antibodies is possible not only against DNA, but against any component of the body during disturbance of homeostasis.

Immunopathological reactions include the reaction to tissue transplantation, in which homologous but transformed substances play the role of antigens which cause cell reactions terminating in necrosis of the transplanted tissue or organ.

The mechanisms of allergy may be considered from the position of immunological genetics. Under the influence of allergens changes occur in the genetic apparatus of the lymphoid cells, which, together with normal globulins and gamma-globulins, produce modified proteins-antibodies.

The pathogenesis of allergy consists of a whole number of damages to the body, e.g., lowering of the dispersity in the humoral medium and occlusion of the capillaries, excitation of the nerve endings and cells of the smooth muscles. Under the effect of the antibody-allergen complex fixed on the surface of the cells a sharp change in metabolic processes and disturbances in life activity of the cells occur. As a result of blocking by the antibody-allergen complex the cells do not receive the necessary substances and as a result excrete an excess of acetylcholine (nerve cells), serotonin (blood cells), heparin (liver cells), and histamine (connective tissue), etc. In small concentrations these substances are necessary for the normal life activity of the body, while in excess they cause dilation of the capillaries and increase vascular permeability, and thus intensify the allergic reaction.

The capillary membranes and smooth muscles are the main sites for manifesting the allergic-anaphylactic reaction. The primary reaction is marked by arterial stasis due to contraction of the arterioles. During asthma the symptoms of disturbances in water metabolism and in the function of the cerebral cortex are observed. During eczema a primary neurovascular process is developed in the skin exhibiting an allergic reaction.

Energy production is preceded by a definite time interval characterized by the accumulation of a sufficient concentration of antibodies, the interaction of which with the allergens is accompanied by the formation of high molecular aggregates consisting of antibodies and allergens. Antibodies which are produced in response to the penetration of allergens into the cells of the macroorganism may enter the blood or lymph or may be fixed on the surface of cell walls.

Depending on where the antibody-allergen complex is formed and localized the development of a definite form of allergic reaction takes place. If the allergen is found in the skin then urticaria appears, whereas if the allergen unites with the antibody in the upper respiratory tract then an allergic rhinitis arises. The formation of an antibody-allergen complex in the eye conjunctiva is accompanied by conjunctivitis, and during localization of the antibody-allergen complex in the mucous membrane of the bronchi an allergic reaction manifests itself as asthma, while the entrance of a certain amount of the antibody-allergen complex into the blood leads to anaphylactic shock.

Of great significance in the development of allergic disease is the condition of the body, the allergic constitution, the degree of excitation of the vegetative nervous system, permeability of the capillaries, and peculiarities of metabolism. In the aetiology of allergy it is impossible to exclude the role of heredity. If the parents are exposed to allergic reactions then they transmit to their children the predisposition to the manifestation of not any one definite form of allergy, but to allergy in general. This predisposition to allergic diseases is transmitted through the blood of the donor suffering from allergic disease.

According to G. Selye, different kinds of diseases of an allergic nature with an increased inflammatory reactivity (rheumatic fever, hay fever, bronchial asthma, allergic skin diseases, etc.) are regarded as diseases of adaptation. They occur as a result of an excessive or an inadequate defense adaptation reaction, and probably are associated with an overproduction of inflammatory hormones of the desoxycorticosterone or aldosterone type in the adrenal cortex.

The frequency of allergy depends on age and intensity of the synthesis of proteins in general, and on the production of antibodies in particular. In newly-born or breast-fed children due to a weak development of the nervous and other systems the phenomena of allergy are encountered comparatively rarely and proceed less intensively. At an age of 18 months and up to the period of puberty allergy is observed considerably more frequently and proceeds more severely. In adults the predisposition to allergic diseases is lowered, and in old age the exposure to allergy becomes slight.

Infectious allergy. In many infectious diseases an increased sensitivity is developed to repeated injections of the causative agent

or the products of its life activity. This state of increased reactivity of the body is known as infectious allergy, and the substances which provoke it, as allergens.

Infectious allergy develops during tuberculosis, leprosy, glanders, brucellosis, tularaemia, actinomycosis, syphilis, dermatomycosis and toxoplasmosis, etc. It can be retained long (for many years) even after recovery. Due to the specificity of allergic reactions they are widely used in the practice of diagnosis of infectious diseases. For this purpose the corresponding allergens are employed which are injected subcutaneously or cutaneously. At the site of injection of the allergen in patients with, for example, tularaemia and brucellosis within 12-48 hours reddening is produced, with swelling and pain. A disadvantage of the diagnostic value of allergic tests is that they may be positive in those inoculated against these diseases and in those who had them many years ago.

* * *

Immunologic reactions include the reactions during transplantation of tissues, in which homologous but transformed substances causing cellular reactions ending in necrosis of the transplanted tissue or organ take part as antigens.

Allergic reactions in some cases occur as a result of the action of many substances having the properties of allergens. Allergens may be substances used in industry, e.g., prolonged contact with dyes, soaps, varnishes, wood, glue, metals, rubber and explosives causes contact dermatitis.

When certain people eat alpine strawberries, strawberries, eggs, milk and crayfish a food allergy develops, called idiosyncrasy, accompanied by skin and intestinal changes.

Drug idiosyncrasy. The side effect of drugs is known as drug or medicamentous idiosyncrasy. It has been known long ago and occurs after the application of a number of preparations (iodine, arsenic, sulphonamides, antibiotics, etc.). Drug idiosyncrasy, according to mechanism of origin of pathological processes, is subdivided into toxic processes occurring as a result of the action on the body of certain chemical groups contained in a drug, allergic reactions appearing due to the increased sensitivity of the body to certain substances, and the state of dysbacteriosis.

Parallergy. Besides strictly specific manifestations of allergy which arise on repeated introduction of one and the same allergen, a nonspecific reaction can be observed which develops in the sensitized body after the effect of substances of another nature. These reactions pertain to the state of parallergy. Nonspecific allergy may occur under the influence of temperature, trauma, radiant energy, chemical substances, etc. The development of nonspecific allergy to a certain extent explains the causes of relapses of tuberculosis and other

diseases accompanied by allergy under the influence of factors nonassociated with allergens. Local sensitization of the portals of entry by the infectious agent in many diseases is of considerable importance.

The Sanarelli and Schwartzman phenomena pertain to nonspecific allergic reactions.

The Sanarelli phenomenon. When a sublethal dose of cholera vibrios is injected into a rabbit's vein, and then a day after a filtrate of colibacillus or *Proteus* is injected in the same way, the rabbit dies within several hours displaying marked symptoms of cholera algid with desquamation of the intestinal epithelium. Further investigations established that rabbits sensitized with cholera vibrios perished from the injection of filtrates of broth cultures of paratyphoid bacteria, staphylococci, streptococci, etc. It proved to be that preliminary sensitization of rabbits may be prepared not only by cholera vibrios, but by various other antigens.

The Schwartzman phenomenon. If a filtrate of enteric fever bacteria is injected intracutaneously into a rabbit and after a few hours this filtrate or the filtrate from other bacteria is injected into the vein, then a haemorrhagic infiltrate develops at the site of intracutaneous injection. The Schwartzman phenomenon, as well as the Sanarelli phenomenon, does not have strict specificity and can be reproduced by different antigens.

The relation of allergy to immunity. The classical point of view presented by R. Koch, C. Pirquet, etc., prevailed for many years in literature, according to which immunity and allergy in tuberculosis were regarded as two states each providing for the other. Later three trends were revealed: the first, classical, in which immunity and allergy were identified; according to the second, immunity opposed allergy, but the latter was given a supplementary defense role in the infectious process; the third trend assessed allergy as a state more likely harmful for the body than beneficial.

The possibility of separating allergy and immunity in tuberculosis has been proved experimentally. It has been established that the distinct condition of active immunity in animals is not harmed after desensitization, i.e., after they have lost their ability to produce an allergic reaction. Consequently, immunity may arise without a simultaneous development of the allergic condition.

Special attention is paid to the works in which it has been shown that in the animal body, found in a state of infectious immunity, spreading of tubercle bacilli proved to be inhibited independent of whether the state of allergy is revealed in the experimental animals or whether it is obviated by desensitization.

The formation and manifestation of barrier-fixing mechanisms of antimicrobial immunity (according to V. Berman) are carried out independent of the presence or absence of the allergic state. Two phenomena which were earlier considered to be indivisible succeeded

in being divided. It was shown that the manifestation of antimicrobial immunity during tuberculosis does not suffer if the visible symptoms of allergy are obviated. This kind of conformity has been established in brucellosis.

Taking into account the high incidence of allergic diseases the necessity arose of working out at a reasonably scientific level important problems such as: regionation (establishing a geographical map) of allergens, diagnostics, therapy and prophylaxis of the pathological processes which they cause.

Among the numerous methods of treating allergic patients, of great significance is the prevention of contact with allergens (foodstuffs, medicinal preparations, household, industrial and other allergens). Sometimes due to the geographical map of allergies it is advisable to change the patient's habitat. Quite favourable results have been obtained by using hormone preparations (ACTH, etc.) which inhibit the process of antibody formation. For lowering the sensitivity of the body and diminishing the susceptibility to the allergen patients are given subcutaneous injections of allergens to which there is an increased sensitivity. This results in the production of blocking (incomplete, monovalent) antibodies which fix the allergens, and thus avert the occurrence of bivalent antibodies capable of combining with allergens and causing the formation of antibody-allergen complexes. The method of desensitization is considered to be effective during allergy caused by household, industrial and plant allergens, and less frequently has a therapeutic action during food and drug allergy. In diseases accompanied by production of a large amount of histamine and other substances in the cells it is advisable to prescribe antihistamine preparations, e.g., dimedrol, pipolphen, suprastin.

Increased sensitivity, according to L. Zilber's opinion, should not oppose immunity, as it is a particular case of immunological processes developing in the body during infection and immunization.

Thus, some authors regard immunity and allergy as two united and indivisible phenomena, whereas others regard them as reactions of different significance for the body, in which immunity is considered to be beneficial and allergy a harmful reaction.

SPECIFIC IMMUNOPROPHYLAXIS AND IMMUNOTHERAPY OF INFECTIOUS DISEASES

VACCINOPROPHYLAXIS

In the general complex of antiepidemic measures great significance is given to specific prophylaxis and therapy of infectious diseases. *Vaccine* (Lat. *vacca*—cow) received its name from the antismallpox preparation prepared from the virus of cowpox. Vaccines are preparations consisting of attenuated or dead causative agents or products of their life activity, while the method of inoculation is known as vaccination or immunization.

Vaccines are introduced into the body cutaneously, subcutaneously, intracutaneously, orally and into the mucous membrane of the nose and pharynx. Within a definite period of time (from several days to several weeks) vaccines give active immunity. Quite strict controls are made on vaccines which should be harmless and highly immunogenic (capable of causing sound and long immunity).

Vaccines are prepared at special factories for biological preparations, institutes for production of vaccines and sera or in separate laboratories at research institutes. Manufactured preparations undergo local and state inspection carried out by special laboratories.

During vaccination the epidemic situation and medical contraindications are taken into account. Vaccination is contraindicated in acute febrile conditions, in convalescence following infectious diseases, in chronic infections (tuberculosis, malaria), heart diseases, severe lesions of the internal organs, the latter half of pregnancy, the first period of breast-feeding and an allergic condition (bronchial asthma, high sensitivity to some foodstuffs), etc.

Vaccines are stored in a dark dry place at a constant temperature (from +2 to +10°C). The term of fitness of the vaccine is stated on the label, and the method of application is described in a special instruction placed inside the box with the bottles or ampoules.

The effectiveness of vaccination depends on the nature and quality of the vaccine, on the correct method of introduction, accuracy of observing the dosage and intervals between administrations, and also on the condition of the people being vaccinated.

The length of postvaccinal immunity varies from several years (vaccine against smallpox, tularaemia) to several months (vaccines against enteric fever, paratyphoid, cholera).

Modern vaccinal preparations are subdivided into 5 groups: (1) vaccines from live causative agents with a decreased (attenuated vaccines) virulence; (2) vaccines from dead cultures of pathogenic microorganisms (bacteria, rickettsiae and viruses); (3) vaccines from the products of chemical cleavage of some bacteria (chemical vaccines); (4) anatoxins received from exotoxins by treating them with formalin at a temperature of 38-40°C; (5) associated vaccines.

Live vaccines include vaccines against smallpox, anthrax, rabies, tuberculosis, plague, brucellosis, tularaemia, yellow fever, influenza, mosquito fever, typhus fever, poliomyelitis, parotitis, measles, etc. Methods of obtaining some vaccines are described in the section "Genetics of Microorganisms", and in the corresponding chapters of "Special Microbiology".

To increase the storage time without loss of immunogenic properties many preparations at present are produced in a dried state. Drying is carried out in a vacuum at a low temperature. Live vaccines are the most effective and valuable preparations, and many of them give long and high-grade immunity. Immunization with live vaccines is the most effective as the body receives a sufficient amount of antigen due to the multiplication of the vaccine strain.

Vaccines prepared from microbes which *have been killed* by heat or by treatment with alcohol, formalin or merthiolate include the enteric fever, paratyphoid, cholera, dysentery, whooping cough, typhus fever, Q fever, tick-borne and Japanese encephalitides, poliomyelitis and leptospirosis vaccines, etc.

Special strains with sufficiently high immunogenic properties are chosen for the preparation of these vaccines.

Chemical vaccines are preparations which are composed not of whole bacterial cells, but of chemical complexes obtained by treating culture suspensions according to special methods.

At present a polyvaccine, consisting of the antigens of the causative agents of enteric fever, paratyphoid, Flexner's and Sonne's dysentery, cholera antigens and a purified concentrated tetanus anatoxin, has been produced, and is used against intestinal infections and tetanus. Bacterial antigens and tetanus anatoxin are adsorbed on aluminium hydroxide.

A chemical associated adsorbed vaccine against intestinal diseases and tetanus has been introduced into practice. It contains O- and Vi-antigens of the causative agent of enteric fever, antigens of the organisms responsible for paratyphoid B, Flexner's and Sonne's dysentery, and concentrated, purified and adsorbed tetanus anatoxin.

Anatoxins are prepared from exotoxins of the corresponding causative agents. Diphtheria and tetanus anatoxins have a wide

application. In recent years an anatoxin has been obtained against gas gangrene. Anatoxins are produced in a purified state; they are freed from impurities and adsorbed into aluminium hydroxide or aluminium phosphate. Anatoxins cause the production of antitoxins and consequently reproduce antitoxic immunity.

The possibility is not excluded of using toxins as prophylactic preparations. At present, a purified, adsorbed toxin intended for immunizing children against scarlet fever is being tested.

Besides the above mentioned preparations *associated vaccines* are used for specific prophylaxis of infectious diseases: whooping cough-diphtheria-tetanus vaccine, diphtheria-tetanus associated anatoxin, whooping cough-diphtheria, diphtheria-whooping cough-scarlet fever vaccines and a tetravaccine composed of enteric fever paratyphoid B, Flexner's and Sonne's dysentery bacteria. Tetravaccines are used in a number of cases together with tetanus anatoxin. Pentavaccine contains enteric fever, paratyphoid A and B and Flexner's and Sonne's dysentery bacteria. It is inoculated together with tetanus anatoxin.

Methods of preparing other associated vaccines are being devised which will provide for the production of antibacterial, antitoxic and antiviral immunity.

Vaccination of the population of the Soviet Union is regulated by health legislation, and it is carried out in a planned manner throughout the whole of the country.

VACCINOTHERAPY

For treating patients with protracted infectious diseases, furunculosis, chronic gonorrhoea, brucellosis, chronic dysentery, etc., vaccines prepared from dead microbes, and anatoxins and antiphagins (staphylococcal extracts obtained by heating, which are filtered and conserved in a 0.25 per cent phenol solution) are used. These preparations include staphylococcal antiphagin, native staphylococcal anatoxin, vaccines—polyvalent (composed of several strains), staphylococcal and streptococcal, polyvalent gonococcal, antibrucellosis, and a vaccine against disseminated encephalitis and multiple sclerosis. In some cases autovaccines are used which are prepared from microbes isolated from patients.

Pyrogenal, a preparation from Gram-negative bacteria, is recommended as nonspecific therapy in inflammatory processes of the eyes and female genitalia, in syphilis of the nervous system, progressive paralysis, eczema, chronic streptoderma, mycoses, different forms of tuberculosis and in many other diseases for increasing the reactivity of the patient and for activation of the functions of organs and systems of the macroorganism.

The mechanism of its action comprises an increase in the permeability of the capillary walls and the main substance of the connective tissue, a stimulation of the function of the hypophyso-adrenal system, an increase in the protein synthesis in the body, an inhibition of the processes of formation of fibroblasts from young cells of the connective tissue, and a decrease in the development of scar tissue.

SEROTHERAPY AND SEROPROPHYLAXIS

The production of sera has been described in the section "Reactions of Immunity and Their Practical Importance" (p. 230). For retaining sterility a preserving substance (chinosol, chloroform) is added to the serum, then it is poured into ampoules which are exposed to local and state inspection. The date of preparation. term of fitness and amount of antitoxic units per 1 ml are stated on the labels. Sera, like vaccines, are stored in a dark, cold place. They are kept from freezing and from the action of high temperatures. They should be transparent, slightly opalescent, and should not produce rough flakes during agitation. The term of fitness of the serum during proper storage is 1-2 years. Dried sera keep considerably longer.

Sera are injected in certain doses intramuscularly, subcutaneously, sometimes intravenously, with strict observation of all the rules of asepsis. A preliminary desensitization according to Bezredka's method is necessary. Sera are employed for treatment and for prophylaxis of tetanus, gas gangrene and botulism. The earlier the serum is injected, the more marked is its therapeutic and prophylactic action. The length of protective action of sera (passive immunity) is from 8 to 20 days.

At present many institutes of vaccines and sera in the Soviet Union produce therapeutic and prophylactic sera in a purified state. They are treated by precipitating globulins with ammonium sulphate, by fractionation, by the method of ultracentrifugation, electrophoresis and enzymatic hydrolysis which allow the removal of up to 80 per cent of unrequired proteins. These sera have the best therapeutic and prophylactic properties, contain the least amount of unrequired proteins, and have a less distinct toxic and allergic action. They are employed in small volumes.

Sera thus produced are subdivided into antitoxic and antimicrobial sera. Antitoxic sera include antidiphtheric, antitetanic sera and sera effective against botulism, gas gangrene, and snake bites.

Antimicrobial sera are used against anthrax, encephalitis and influenza.

Gamma-globulins are obtained by the method of fractionation of serum proteins by means of alcoholic solutions at temperatures

lower than 0°C. The separation of protein fractions is based on their various solubility during changes in alcohol concentration, pH, and electrolytic content. Methods have been devised to obtain stable, electrophoretically pure preparations of gamma-globulin with an 8-10 per cent yield of the total protein serum.

Gamma-globulins are used for prophylactic purposes against measles, poliomyelitis, whooping cough, epidemic hepatitis, and smallpox. Gamma-globulin is used together with antirabic vaccine against rabies. Gamma-globulins are completely harmless preparations, and they do not contain the virus of epidemic hepatitis and causative agents of other diseases.

CHEMOTHERAPY AND CHEMOPROPHYLAXIS OF INFECTIOUS DISEASES

CHEMOPREPARATIONS

In medical practice besides vaccines and sera for treating patients with infectious diseases and in some cases for prophylaxis various chemical substances comparatively harmless for the macroorganism are widely used which have a lethal action on pathogenic microorganisms.

This method was known long ago to ancient people, and was used for the treatment of some diseases. The Peruvian Indians discovered the therapeutic action of cinchona bark, and in the 18th century cinchona bark was brought to Europe. The inhabitants of Brazil successfully employed the root of the ipecacuanha for treating amoebiasis. Mercury has been extensively employed in the therapy of syphilis. In the middle of the 16th century this method became known to the people of Europe.

The basis of modern chemotherapy was founded by P. Ehrlich and D. Romanowsky, who formulated the main scientific principles and the essence of chemotherapy. They showed that in the treatment of each infection a substance should be found which, during injection into the diseased body, will bring the least harm to it and cause the most destructive action to the pathogenic agent (causative agent).

P. Ehrlich devised the principles of synthesis of medicinal substances by chemical variations: methylene blue, derivatives of arsenic—atoxyl, salvarsan ("606"), neosalvarsan ("914"). By the further development of chemistry new medicinal preparations could be obtained.

Extensive experimental and clinical tests of chemopreparations were carried out by E. Metchnikoff.

Chemopreparations should have a specific action, a maximal therapeutic effectiveness, and a minimal toxicity for the body.

As a characteristic of the quality of a medicinal preparation, P. Ehrlich introduced *the chemotherapeutic index* which is the ratio of the maximal tolerated dose to the minimal curative dose:

$$\frac{\text{maximal tolerated dose/DT—Dosis tolerata}}{\text{minimal curative dose/DC—Dosis curativa}} > 3.$$

The chemotherapeutic index should not be less than 3.

The mechanism of action of chemicals. Chemotherapeutic substances do not have the properties of sterilizing the body. At the basis of bacteriostatic action of chemotherapeutic compounds lies the disturbance of the processes of biological synthesis in the microbial cell, as a result of which the cell ceases to obtain material for growth and reproduction.

P. Ehrlich (1854-1915)

Chemotherapeutic preparations include a number of compounds used in medicine.

Arsenic preparations (novarsenol, myarsenol, aminarsone, osarsol, ets.) are administered in syphilis, relapsing fever, trypanosomiasis, amoebiasis, balantidiasis, anthrax, sodoku and other diseases.

Bismuth preparations (basic bismuth nitrate, xeroform, basic bismuth salicylate, bioquinol, bismoverol, bithiurol, pentabismol, etc.) are used against enterocolitis and syphilis.

Antimony compounds (tartaric antimony potassium salt, stibenil, stibozan, surmine, solusurmine, etc.) are used for treating patients with leishmaniasis and venereal lymphogranulomatosis.

Mercury preparations (mercury salicylate, mercuric iodide, mercury cyanide, calomel, unguentum hydrargyri cinereum containing metallic mercury, etc.) are prescribed for treating patients with syphilis and are used as antiseptics in pyogenic diseases.

Acridine preparations (rivanol, tripaflavine, acriflavine, acricide, flavicid, etc.) are recommended for pyogenic diseases and inflammatory processes of the pharynx and nasopharynx.

Antimalarial substances include more than 50 preparations, e.g., quinine hydrochloride, quinine sulphate, acrichine, plasmocide, bigumal, chloridine, cyclochine, resochine, quinocide, etc.

Alkaloid preparations (emetine, etc.) are used for treating patients with amoebiasis.

Sulphonamide preparations. The introduction into practice of compounds of the sulphonamide group (streptocid, ethasole, norsulphazol, sulphazine, methylsulphazine, sulphadimezin, urosulphen, phthalazole, sulgine, sulphacyl, soluble sulphacyl, disulphormin, etc.) marked a revolution in the chemotherapy of bacterial infections.

Sulphonamide preparations are used during pyogenic diseases, tonsillitis, scarlet fever, erysipelas, pneumonia, dysentery, gas gangrene, gonorrhoea, cystitis, venereal lymphogranulomatosis, psittacosis, ornithosis, trachoma, blennorhoea in the newborn, etc.

There are several points of view concerning the mechanism of action of sulphonamides on microbes.

1. D. Woods' theory regards sulphonamides as analogues of para-aminobenzoic acid which compete with the latter for the possession of the specific protein of an enzymatic nature, necessary for the growth of the microorganism. The formation of new "unnatural" protein components leads to growth inhibition.

2. According to P. Fildes' theory the para-aminobenzoic acid is the necessary metabolite of the bacterial cell, which plays an important part in the growth of the latter, and not the component part of some enzyme. Sulphonamides and para-aminobenzoic acid act in a mutually inhibiting manner, while a lack of the metabolite leads to a cessation of growth inhibition.

3. The enzymatic theory of M. Sevaga considers that para-aminobenzoic acid plays a less important role. According to this theory, the sulphonamides due to structural similarity with coenzymes inhibit the action of desmolysing enzymes of the bacterial cell, taking part in all reactions which supply the energy necessary for the growth and division of the cell.

New **synthetic preparations**: PAS, tibone, phthivazide, isoniazid, saluzid, metazid, larusan, etoxid, sulphonin, uglon, crisanol, etc., are used for treating tuberculosis patients. Of these phthivazide which is a derivative of isonicotinic acid hydrazide has a good therapeutic action.

There are related phthivazide preparations which are manufactured under the following names: isoniazid, rimifon, nidrizid, marsilid, neoteben, nicotidin, bacillin, amitazan, phtizen, artuban, etc.

Isoniazids easily penetrate the macrophages and have a bacteriostatic action on tubercle bacilli phagocytized by these cells.

The mechanism of the action of phthivazide and isoniazid is as follows. The metabolism of the tubercle bacilli is impaired and compounds are formed similar in chemical structure to the metabolite of the bacterial cell but physiologically inert, which block the substance associated with bacterial multiplication.

Under the influence of isoniazid tubercle bacilli lose their acid-fastness. Isoniazid-stable bacteria lose their virulence. Isoniazid causes a change in cultural properties and oxybiontic processes.

Antiblastoma preparations. From a large number of preparations which have been obtained only a few have found application in clinical practice. Sarcolysin is used in seminoma, myeloma, and bone tumours. Thiophosphamid (Thio Tef) is effective in ovarian tumours and breast cancer. For treating lymphogranulomatosis

degranol, nitromin, K-39, vinblastin, endoxan, etc., are recommended. Dopan, endoxan, dipin, myelosan (myleran), 6-mercaptopurine, metatrexat, and vincristin are prescribed against acute and chronic leukoses. The best of them is dopan which gives fewer complications and can be administered orally. The above mentioned preparations do not have a good therapeutic action, and some of them inhibit the haematopoietic system, cause mutations of healthy cells, and can cause new cancerous diseases.

ANTIBIOTICS

Antibiotics (Fr. *anti*—against, *bios*—life) are chemical substances excreted by some microorganisms which inhibit the growth and development of other microbes (in recent years several antibiotics have been obtained synthetically and semisynthetically).

Ch. Darwin began scientific investigation into the problems of natural selection and interspecies struggle. Antagonistic interrelations between microorganisms of various species were first observed by L. Pasteur in 1887. He established that anthrax bacteria die rapidly in mixed cultures with putrefying bacteria, and he characterized this phenomenon as a struggle for existence.

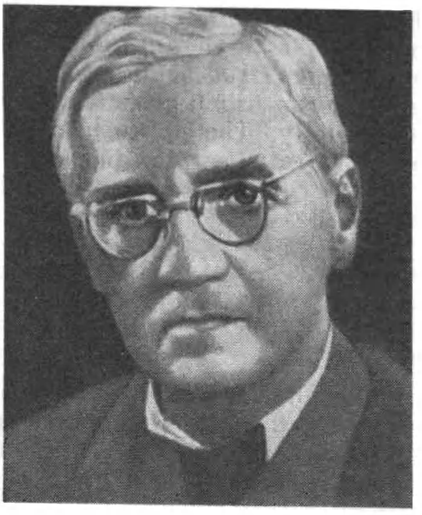
The main causes for this antagonism may be competition for oxygen or nutrient substances, the excretion into the cultural medium of acid or basic substances inhibiting growth, and the accumulation of chemical substances with the help of which some species of microbes inhibit the growth of others.

The essence of this phenomenon is that in the process of evolution of plant and animal organisms the most varied and subtle adaptations were formed, which reflects the general biological law of the struggle for existence. The latter, as E. Metchnikoff pointed out, has a more universal nature, should be applied to microbes, and can be used for treatment and prophylaxis of infectious diseases in animals and humans. E. Metchnikoff is the founder of the study of antagonism in microbes and of the practical use of this phenomenon. He employed lactic acid bacteria for inhibiting harmful microflora of the intestine.

In 1871-72 V. Manassein and A. Polotebnov were the first to use the therapeutic properties of fungi from the genus *Penicillium*. The first antibiotic of bacterial origin employed as an antiseptic was obtained from the blue pus bacteria by R. Emmerich and O. Low and was named pyocyanase. In 1887 N. Gamaleia extracted an antibiotic preparation—pyoclastin from a culture of blue pus bacteria. A. Pavlovsky (1887) by injecting the *Bacterium prodigiosum* and other bacteria into a rabbit's body infected with anthrax bacilli succeeded in not only protecting the animal from diseases, but also in curing the latter. V. Sirotinin (1888) established the antagonistic action of anthrax bacillus on enteric fever bacillus, and N. Blagoveshchensky (1890) determined the antagonistic effect of blue pus bacteria on the anthrax bacillus. S. Vinogradsky observed the phenomenon of antagonism in soil microbes. In 1904 M. Tartakovsky used a green mould against the microbes which cause diseases in chickens.

In 1909 Lashchenkov and in 1922 A. Fleming isolated the enzyme lysozyme which is capable of inhibiting a number of microorganisms.

However, the study of antibiotics began in 1928, when A. Fleming proved that the filtrate of a broth culture of the fungus *Penicillium notatum* has antibacterial properties in relation to Gram-positive microorganisms. In 1940 E. Chain and H. Florey obtained a relatively stable preparation of penicillin. In 1942 Z. Ermolyeva prepared penicillin from *Penicillium crustosum*.



A. Fleming (1881-1955)

Further development of this problem is associated with the works of various scientists: R. Dubos isolated gramicidin and tyrocidin from the cultural liquid of *S. brevis*; S. Waksman and co-workers devised a method of producing streptomycin; B. Tokin discovered antimicrobial substances from plants—phytoncides, and others who enriched modern medical practice with numerous preparations widely used for the treatment of infectious diseases.

Antibiotics are obtained by special methods employed in the medical industry. For the production of antibiotics strains of fungi, actinomycetes, and bacteria are used, which are seeded in a nutrient substrate. After a definite growth period the antibiotic is extracted, purified and concentrated, checked for innocuousness and potency of action.

In composition a number of antibiotics (penicillin, streptomycin, gramicidin, etc.) have optically distorted molecules. The antibacterial properties of some antibiotics are associated with optical inversion of their molecules which have the same physicochemical properties as normal molecules and can easily be bound to the enzime. Since they lack the ability to participate in biochemical reactions, this binding is accompanied by a blockage of enzymes, and consequently, a growth inhibition followed by death of the microorganism.

A characteristic property of antibiotics is the bacteriostatic and bactericidal action on microbes. Each antibiotic is characterized by a specific antimicrobial spectrum of action (Fig. 75). Some antibiotics are inactivated in the presence of animal and plant proteins. Only a few antibiotics have a powerful antibacterial action, which does not decrease in the presence of protein matter of animal tissues and at the same time is not toxic (in certain concentrations) for the human being.

The mechanism of action of antibiotics varies. For example, penicillin inhibits the synthesis of polymers of the bacterial cell wall (it hinders the use of muramic acid by bacteria), which leads to an increase of cells incapable of multiplication. Sometimes the action of penicillin leads to the formation of L-forms in the shape of pleomorphic protoplasmic structures. Thus, penicillin has a

lethal effect not on the given population, but on its offspring. The selective action of penicillin on microbes hinders the penetration of glutamic and other amino acids through the cytoplasmic membrane of pathogenic cocci unable to synthesize amino acids which are vitally important for the existence of these bacteria. Penicillin inhibits the ability of the bacterial cell to absorb protein components—amino acids, and it inhibits the synthesis of the enzyme system and also of adaptive enzymes.

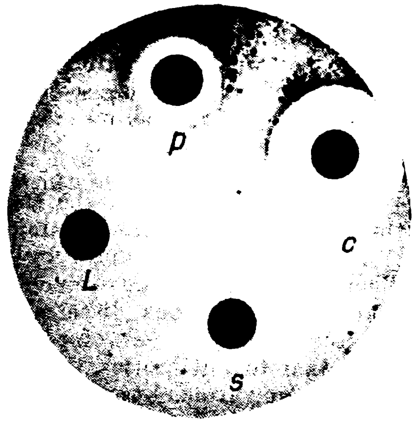


Fig. 75. Inhibition of the growth of staphylococcus under the influence of penicillin (p), levomycetin (l), streptomycin (s), chlortetracycline (c)

Due to the widespread use of penicillin many species of pathogenic microorganisms have become resistant to this antibiotic. Resistance in bacteria to penicillin may depend on (1) the formation of extracellular penicillinase by bacteria; (2) the destruction of penicillin inside the cell not necessarily by penicillinase; and (3) low activity of the penicillin-binding component.

Streptomycin causes an inhibition of the incorporation of some amino acids in protein synthesis and inhibits the transport of amino acids from RNA to the protein molecule. Streptomycin attacks the bacterial enzyme in the presence of which the introduction of pyruvic acid into the tricarbonic acid cycle by its union with oxalacetic acid takes place. This antibiotic inhibits the activity of biotin-containing enzymes catalyzing the union of carbon dioxide with carbonic acids.

Of special interest is the mechanism of action of streptomycin on tubercle bacilli. This preparation does not have a sterilizing action, but inhibits the respiration of tubercle bacilli, which leads to the inhibition of cell reproduction and toxin formation. At the same time stimulation of tissue respiration occurs in the patient as well as an increase in the ability of the macroorganism to destroy tubercle bacilli and their toxins.

The selective action of streptomycin on the tubercle bacillus is due to the fact that the permeability of cell membranes in the bacilli and in the tissue cells of animals and man differ due to the dissimilar chemical structure of the cytoplasm of these organisms.

There are data showing that streptomycin inhibits the capacity of bacterial cells of the colibacillus to oxidize fumaric and glutamic acids. This leads to an inhibition of adaptive enzyme production.

In several investigations it has been established that chloromycetin (chloramphenicol, levomycetin) suppresses protein synthesis and the assimilation of ammonia, inhibits the absorption of oxygen, hinders the incorporation of amino acids into proteins of certain fractions, etc.

Tetracycline stops the synthesis of proteins, nucleic acids and the cell wall.

The effectiveness of antibiotics also depends on their ability to bind with various blood proteins of the tissue of the macroorganism, and to be transported into the sites of localization of the causative agents of infectious diseases. Some antibiotics (penicillin, streptomycin, etc.) are bound better with albumins, while others (tetracycline, albomycin, etc.) combine more succesfully with globulins.

As has been affirmed, the best studied reason for the decline in the therapeutic action of antibiotics is considered to be the formation of adaptive enzymes by pathogenic bacteria, which break down antibiotics. To overcome the difficulties involved during therapy of infectious diseases attempts have recently been made to use antienzyme sera for neutralization of the enzymes which are lethal to antibiotics.

There are various hypotheses and theories which have not entirely revealed the mechanism of action of antibiotics, and this question has not been completely solved.

Investigations have established that most antibiotics do not kill pathogenic microbes which penetrate into the body of the man or animal and only inhibit their development and weaken their life activity. The freeing of the body from microbes harmed by antibiotics is performed by defense adaptations of the macroorganism.

The activity of antibiotics is expressed in international units (IU). Thus, for example, 1 IU of penicillin (Oxford unit) is the smallest amount of preparation inhibiting the growth of a standard *Staphylococcus aureus* strain. Recently the method of determining the activity of antibiotics according to the weight of the preparation has received wide application.

One unit of activity (AU) corresponds to the activity of 0.6 micrograms (μg) of the chemically pure crystalline sodium salt of benzylpenicillin. Consequently, in 1 mg of sodium salt of benzylpenicillin there may be 1,667 AU, and in 1 mg of potassium salt — 1,600 AU. For practical purposes both preparations are manufactured with an activity not less than 1,550 AU.

The concentration of dry preparations as well as solutions is expressed as the number of micrograms of active substance in 1 g of preparation or in 1 mg of solution.

According to their origin, all antibiotics are subdivided into three groups: antibiotics of an animal origin, antibiotics of a plant origin, and synthetic and semisynthetic antibiotics (Table 13).

Table 13

Classification of Antibiotics According to Origin

Antibiotics of an animal origin	Antibiotics of a plant origin				Synthetic and semisynthetic antibiotics
	produced by higher plants	produced by fungi	produced by actinomycetes	produced by bacteria	
Lysozyme Erythrin Ecmolin Leukin Plakin Interferon, etc.	Phytoncides: allicin, raphanin, imanin, etc.	Penicillin and penicillin-like preparations	Streptomycin Chloromycetin (chloramphenicol) Chlortetracycline (aureomycin, biomycin) Oxytetracycline (terramycin) Erythromycin Albomycin Neomycin Nystatin, etc.	Gramicidin Polymyxins A and M, etc.	Synthomycin Levomycetin Sanasine Phenoxymethyl penicillin Methycillin Broxil Selbinin Penbritin, etc.

Antibiotics of animal origin. Lysozyme was obtained from egg white. It is a polysaccharide resistant to heat and to the action of acids. Lysozyme was discovered in the spleen, heart, liver, lungs, in various secretions (lacrimal, nasal mucus, saliva, etc.), and also in egg white, and in the juices of some plants.

Lysozyme inhibits the growth of various saprophytic bacteria usually isolated from the air. It is a special antibacterial substance and plays a particular role in natural immunity of the animal organism. Lysozyme hydrolyses the bonds coupling the carbohydrate components with the component part of the glucoprotein molecule. Lysozyme has not received widespread medical application, as its action is not lethal to many pathogenic microorganisms.

Antibiotics of an animal origin include ecmolin, erythrin, leukin, plakin, ingibin, tuberculostatic factor and other substances capable of suppressing or inhibiting the growth of different bacterial species.

The cells of some species produce interferon which inhibits the life activity of causative agents of many viral infections.

Antibiotics of plant origin. Phytoncides (Gr. *phyton*—plant, Lat. *caedere*—kill) are volatile substances which are excreted by plants and have an antibiotic action.

1. *Allicin* is obtained from garlic (*Allium sativum*). It inhibits the growth of Gram-positive and Gram-negative bacteria. Methods of preparing a stable and effective medicinal preparation from garlic heads are being devised. Garlic infusions, allylsate, allylchep, and allylglycer are employed for suppressing putrefying processes in the intestine and during colitis.

2. *Raphanin* is obtained from radish (*Raphanus sativus*). It acts on Gram-positive and Gram-negative bacteria in a 1:100 dilution.

3. *Imanin* is obtained from St. John's wort (*Hypericum perforatum*). It is used for treating purulent processes and severe burns.

Antibiotics produced by fungi. Penicillin is produced by the fungi *Penicillium notatum* (Fig. 76*a*), *Penicillium chrysogenum*

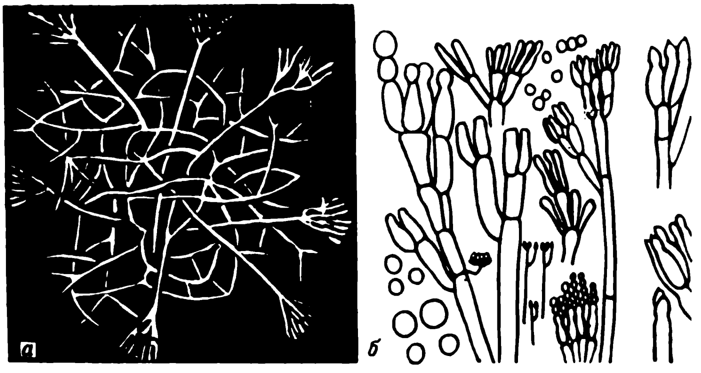


Fig. 76. *Penicillium notatum* (*a*), *Penicillium chrysogenum* (*b*)—producers of penicillin

(Fig. 76*b*), etc. Penicillin is produced as sodium and potassium salts. It dissolves readily in water, but its solutions are not stable. It is a dipeptide consisting of dimethylcisteine and acetylserine.

Penicillin is used in staphylococcal, streptococcal, pneumococcal and meningococcal infections, gas gangrene, gonorrhoea, syphilis, leptospirosis, anthrax and other diseases.

Penicillin preparations include ecmonovocillin which is a form of long-acting penicillin, maintaining the necessary therapeutic concentration of penicillin in the blood. It is used only for intramuscular injections. The indications are the same as for the application of penicillin.

New drug forms of penicillin have been obtained, e.g., bicillin-1, bicillin-3, which are retained for long periods in the body. The former is an *N*, *N'*-dibenzylethylenediamine salt of benzylpenicillin, the latter—a mixture of novocain, potassium salt of benzyl-

penicillin and bicillin-1; and ephicillin (hydrogen iodide salt of diethylaminoethyl benzylpenicillin).

One product of the life activity of penicillium is griseofulvin obtained in 1939 from mycelia of *Penicillium griseofulvum.* At present griseofulvin is manufactured by the medical industry. It is obtained by the deep method of fermentation of *Penicillium nigricans.* It is employed for treating trichophytosis, microsporosis, epidermophytosis and favus.

Microcide is produced by *Penicillium vitale.* It is used externally in recently infected wounds and in other acute purulent processes. It leads to a rapid cleaning of wounds and ulcers from pus, and to a decrease in inflammatory phenomena. It is used for treating patients with burns, frost-bites, infected and unhealable wounds and ulcers.

Antibiotics produced by actinomycetes. 1. *Streptomycin* is obtained from *Streptomyces griseus* (Fig. 77). Chemically it consists of two components: the nitrous base of streptidin and streptobiosamine. Streptomycin is a base and forms salts with acids, which readily dissolve in water and are insoluble in organic solvents. It has a bacteriostatic property in relation to Gram-negative as well as to Gram-positive pathogenic microbes.

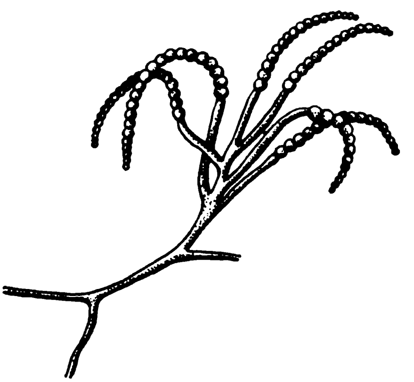


Fig. 77. *Streptomyces griseus*—producer of streptomycin

Streptomycin has a good therapeutic action on tuberculosis, tuberculous meningitis, plague, brucellosis, tularaemia, whooping cough, etc.

New salts of streptomycin have been obtained, e.g., pasomycin (para-aminosalicylic salt of dihydrostreptomycin) which has proved to be effective in relation to streptomycin-resistant tubercle bacilli; dihydrostreptomycin pantothenate (pantothenic salt of dihydrostreptomycin) and streptomycillin (combined medicinal form of penicillin and streptomycin).

2. *Chloromycetin* (chloramphenicol) is obtained from the cultural fluid of a strain of *Streptomyces venezuelae*, isolated from the soil in tropical South America. It has a good therapeutic effect during dysentery, enteric fever, typhus fever and other rickettsioses.

3. *Chlortetracycline* (biomycin, aureomycin) is produced by *Streptomyces aureofaciens.* It is employed during staphylococcal infections, pneumonia, subacute septic endocarditis, rickettsioses, amoebiasis, dysentery, whooping cough, gonorrhoea, brucellosis, tulara-

emia, trachoma, psittacosis, peritonitis, surgical sepsis and other diseases.

4. *Tetracycline* is a derivative of chlortetracycline. It is obtained by reductive dechlorination of chlortetracycline. Tetracycline has a wide spectrum of action, it inhibits many species of Gram-positive, Gram-negative and acid-fast microbes. It also inhibits the development of many rickettsiae, large viruses and some protozoa. It is used in treating patients with pneumonia, subacute septic endocarditis, amoebiasis, dysentery, whooping cough, gonorrhoea, in diseases of the urogenital tract, typhus fever and other rickettsioses, and for the prevention of suppurative processes in surgery. Tetracycline hydrochloride is manufactured in the form of pills with pure tetracycline or in combination with nystatin.

5. *Oxytetracycline* (terramycin) is obtained from *Streptomyces rimosus*. In spectrum and mode of action it is close to chlortetracycline.

6. *Erythromycin* is obtained from *Streptomyces erythraeus*. It is administered in streptococcal and pneumococcal diseases. In experiments on animals it has proved to be effective in diseases caused by Gram-positive and Gram-negative bacteria, rickettsiae, chlamydias, intestinal amoebae and trichomonads. Diphtheria bacilli are quite sensitive to erythromycin.

7. *Albomycin* is obtained from *Streptomyces subtropicus*. It is given orally in pneumonia and septic diseases in children, in diseases caused by Gram-positive and Gram-negative bacteria which are resistant to other antibiotics. A good result has been observed in treating staphylococcal and pneumococcal infections, sepsis, peritonitis and endocarditis.

8. *Neomycin* has been isolated from *Streptomyces fradiae*. It has a bacteriostatic action against Gram-negative and Gram-positive bacteria. The preparation is slightly toxic. It is prescribed mainly for the local treatment of suppurative processes, caused by staphylococci which are resistant to penicillin and to other antibiotics, and also during colienteritis, the causative agents of which are the pathogenic serotypes of *E. coli*.

9. *Nystatin* has been extracted from the cultural fluid of *Streptomyces noursei*. It inhibits many pathogenic fungi and some pathogenic protozoa. It is nontoxic when used per os. It has received wide application in treatment of candidiasis.

10. *Kanamycin* is an antibiotic produced by *Streptomyces kanamyceticus*. In mode of action it resembles streptomycin and neomycin. It inhibits the growth of Gram-positive and Gram-negative bacteria. Kanamycin is used for treating patients with tuberculosis in whom the causative agent became resistant to antituberculous chemopreparations and antibiotics. It is prescribed for treating anthrax, gonorrhoea and for acute and chronic forms of infections of the urinary tract, and diseases caused by resistant strains of

staphylococci. Kanamycin is used in preoperative preparation (the intestine.

11. *Monomycin* is a preparation which in biological and chemica properties resembles kanamycin. It is less toxic than neomycin It has been isolated from *Actinomyces circulatus*. Good result have been obtained in treating patients with dysentery and colien teritis, caused by pathogenic serotypes of *E. coli*, and in toxic dys pepsias of an unrecognized aetiology.

Antibiotics produced by bacteria. 1. *Gramicidin* isolated from culture of *B. brevis* has a bacteriostatic and bactericidal actio on some pyogenic cocci.

2. *Soviet gramicidin* (gramicidin C) is produced by a specia subspecies of *B. brevis*. It is easily crystallized and has a wide spectrum of action. It is used in treating pyogenic diseases, ero sions of the cervix uteri and colpites, ulcerous affections of the mucous membrane in dysentery, and in gas gangrene. It stimulate phagocytosis. It is used as an antiseptic in surgery.

3. *Polymyxins A and M* are produced by *Bac. polymyxa*. They are prescribed in diseases caused by Gram-negative bacteria. Polymyxin B (foreign) and polymyxin M (Moscow) are known.

Antibiotic substances include *bacteriocins* produced by various strains of *E. coli*, *E. freundi*, *Salmonella* and *Shigella*. Bacteriocins are capable of inhibiting the growth of certain strains of bacteria of the same species. At present many antibiotic substances produced by bacteria have been described. The production of bacteriocins takes place as a result of recombination. The mechanism of action is unrecognized. They have bactericidal properties. Bacteriocins, similar to temperate bacteriophages, have the properties of episomes. Up till now a practical application of bacteriocins has not been found, but they are an important antibacterial factor in combating pathogenic microbes.

Synthetic and semisynthetic antibiotics. Methods of obtaining antibiotics synthetically have been devised. These synthetic preparations include synthomycin and levomycetin which are substitutes for chloromycetin, the natural product of the life activities of *Streptomyces venezuelae*. It has been possible to synthesize racemic chloromycetin, synthomycetin, and the laeotropic isomer, levomycetin. These preparations are successfully used for treating patients with dysentery and especially children with dyspepsia. They are successfully used in rickettsioses, tularaemia, whooping cough, gonorrhoea, trachoma, erysipelas, enteric fever, brucellosis and coccal diseases which do not yield to treatment with penicillin.

In 1946 penicillin was obtained synthetically, and in 1957 the acid-fast phenoxymethyl penicillin, broxil, selbenin, penbritin, semisynthetic tetracyclines and other preparations were synthesized.

Synthetic preparations include *sanazin*—the homologue of the antibiotic pyocyanin. It has an antimicrobial action and inhibits

the growth of and kills trichomonads, staphylococci, haemolytic streptococci, and the causative agents of tuberculosis, whooping cough, dysentery, cholera, diphtheria, etc. It is used for treating tuberculosis of the eye and tuberculosis of the bones and joints, and also for the treatment of carriers of diphtheria bacilli and haemolytic streptococci.

Antibiotics include preparations such as magmomycin, puromycin, vitacycline (tetracycline with vitamins C, B_1 and B_2), oleotetrin (oleandomycin), sigmamycin (combined preparations consisting of tetracycline and oleandomycin) and many others.

New medicinal forms of tetracyclines having weaker side effects have been devised.

Due to the wide distribution of staphylococci resistant to antibiotics a search for new preparations became necessary. At present a semisynthetic staphylococcal penicillin has been obtained which has a distinct bacteriostatic action on resistant strains of pathogenic staphylococci. With the isolation of the penicillin nucleus, 6-aminopenicillanic acid (6APA), it became possible to obtain various derivatives of penicillin: phenoxyethyl-, phenoxypropyl-, phenoxybenzylpenicillin, etc.

Dimethylchlorotetracycline from the group of tetracyclines has been put into production in the USA. It is used for the treatment of many infectious diseases and in doses half as strong as tetracycline. A good result has been obtained in treatment of inflammatory processes of the urinary tract.

With the discovery of the antibiotic griseofulvin dermatology was enriched with an effective preparation with the help of which diseases of the skin, hair and nails caused by fungi imperfecti could be treated.

The number of new antibiotics increases every year. In 1964 the preparations numbered 1,800, more than 50 of which were produced at industrial enterprises. Special attention has been given to the problems of obtaining antibiotics by synthetic methods. Synthetic and semisynthetic preparations have a high therapeutic effect and a wide spectrum of action.

Some antibiotics have a poisonous effect on rats, insects and mites. They are used for exterminating rodents and arthropods, the vectors of infectious diseases. Antibiotics are widely used in the conservation of foodstuffs.

Antibiotics (kormogrisin, chlortetracycline, etc.) stimulate the growth of animals and fowl, and are therefore widely used in agriculture.

Of interest is the very difficult problem of chemotherapy of viral diseases. At present there are no effective drugs against viral infections. This is due to the biological peculiarities of viruses as obligate intracellular parasites, which must be acted upon by other means than those used in microbial diseases.

However, in spite of the great difficulties, substances have been found (actinomycins, mytomycins) which are capable of inhibiting the synthesis of the viral components without impairing the life processes of the cell. Interferon which is being tested against a number of viral diseases (see p. 227) has good prospects.

In recent years many new antibiotics have been obtained which have a good effect in the treatment of murine leukoses. Some of them are employed successfully in agriculture for treating fowl leukoses. Only rare preparations are used for therapy of leukosis (myeloleukosis) in hospitals.

Antitumour antibiotics include actinomycins C,D,K,F, etc., carcinophilin, mytomycin, actinoxanthin, chrysomalin, aurantin, sarcomycin, neocid and krucin. Their therapeutic effectiveness is small. They are all toxic.

Side effects of antibiotics. It has been established that large doses of penicillin and streptomycin have a neurotoxic action, tetracyclines affect the liver, chloromycetin has a toxic effect on the haematopoietic organs, and chlortetracycline and oxytetracycline upon intravenous injection may lead to collapse with a lethal outcome. Upon injection of penicillin and streptomycin a rash, contact dermatitis, angioneurotic oedema, anaphylactic reactions or allergic asthma may occur. Quite frequently allergic reactions arise during local application of antibiotics. Of the most practical importance is their indirect action which is mainly due to the development of resistant strains of microorganisms, sometimes causing furuncles or severe generalized diseases which develop vigorously, in some cases with a lethal outcome. In cases of the application of antibiotics with a wide spectrum of action infections may develop which are caused by resistant strains of *Proteus* and fungi.

Staphylococcal colitis proceeds very severely, and is characterized by profuse diarrhoea, dehydration of the body, toxic phenomena, shock and collapse.

Of great hazard is the formation of resistant staphylococci which cause various postoperative complications—persistent furunculosis and staphylococcal septicaemias.

The most severe complication is anaphylactic shock from the use of penicillin in which a rapid drop in blood pressure, cyanosis, superficial breathing, loss of consciousness, and convulsions are observed, and in some cases death occurs. Complications caused by penicillin are characterized by allergic reactions and proceed according to the serum sickness type.

In prolonged use of penicillin or levomycetin (in syphilis and enteric fever) collapse is one of the severe side effects.

Contact dermatitis is an allergic reaction of a medicinal origin. This disease is caused by the action of streptomycin in medical personnel and patients using this preparation over long periods. Quite often allergic manifestations are recorded in the mucous

membranes such as hyperaemia and oedema of the pharynx and tongue. In children antibiotics with a wide spectrum of action cause perianal skin hyperaemia, and hyperaemia of the rectal mucosa.

In some countries the number of cases of infection with staphylococcal pneumonia in children has increased. It has been suggested, that this can be explained partly by the origin of penicillin-resistant strains of staphylococci. The disease has the tendency of becoming complicated with abscesses, empyema, pneumothorax and the formation of cysts.

Prolonged use of antibiotics may cause vitamin deficiency. Many analogues of para-aminobenzoic acid are capable of inhibiting the growth of bacteria and at the same time cannot be used as medicinal preparations, since most of the blocked bacterial vitamins are essential for the human body.

Searches are being made for antibacterial preparations which are capable of blocking bacterial vitamins only, and not disintegrating the vitamins of the macroorganism.

Due to the wide distribution of antibiotic-resistant pathogenic bacteria the use of combined treatment with new antibiotics, to which causative agents of infectious diseases have not become accustomed, has been recommended. For the purpose of averting the development of resistant forms of microbes combined preparations are prescribed, such as penicillin-streptomycin, erythromycin-oxytetracycline, etc. Tetracycline with nystatin is used for the prevention of candidiasis.

It has been established that treatment with antibiotics in a number of cases arrests the production of immunity and may even hinder this process.

Thus, antibiotics as highly effective medicinal preparations should be used rationally, taking into account their side effects.

PART THREE

*

SPECIAL MICROBIOLOGY

Translated by L. Aksenova

PATHOGENIC COCCI

The pathogenic cocci include staphylococci, streptococci, pneumococci, meningococci, and gonococci. They cause inflammatory processes in human beings, with the formation of pus. For this reason they are known as suppurative (pyogenic) cocci.

There are several types of symbiosis between cocci and the human body. Saprophytic and conditionally pathogenic types of staphylococci and streptococci live on the skin, mucous membranes, and in the respiratory tract. Meningococci may be harboured for long periods in the nasopharynx, and faecal streptococci (enterococci), in the intestines. When body resistance is lowered or the skin and mucous membranes are injured, these bacteria penetrate the body tissues and cause infection.

The various pathogenic cocci possess different organotropic ability. This is distinctly manifest in pneumococci, meningococci, and gonococci but less so in staphylococci and streptococci.

Cocci belong to the order *Eubacteriales*, and families *Micrococcaceae*, *Lactobacillaceae*, and *Neisseriaceae*.

STAPHYLOCOCCI

The pathogenic staphylococcus, *Staphylococcus aureus*, was discovered by R. Koch (1878), and later isolated from furuncle pus by L. Pasteur (1880). It has been described as the causative agent of numerous suppurative processes by A. Ogston (1881), and has been studied in detail by F. Rosenbach (1884).

Morphology. Staphylococci are spherical in shape, 0.8-1μ in diameter, and form irregular clusters resembling bunches of grapes (Fig. 78). In smears from cultures and pus the organisms occur in short chains, in pairs, or as single cocci. Large spherical (L-forms) or very small (G-forms) and even filterable forms may be seen in cultures which have been subjected to various physical, chemical, and biological (antibiotics) factors. Staphylococci are Gram-posi-

tive organisms which possess no flagella and do not form capsules or spores (see Fig. 117, *1*). In old cultures certain cells are found to be Gram-negative.

Cultivation. Staphylococci are aerobes or facultative aerobes. They grow well on ordinary nutrient media with a pH of 7.2-7.4 at a temperature of 37°C but do not grow at temperatures below 10° and above 45°C. At room temperature with adequate aeration and subdued light the organisms produce golden, white, lemon-yellow, and other pigments known as lipochromes. These pigments do not dissolve in water but are soluble in ether, benzene, acetone, chloroform, and alcohol. They are most readily formed on milk agar and potatoes at a temperature of 20-25°C.

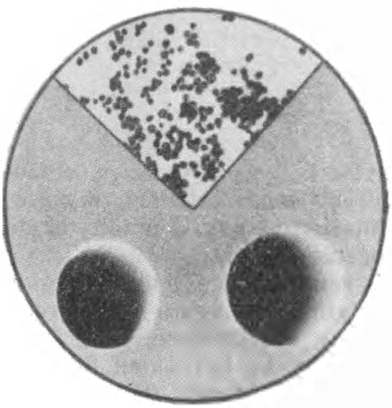


Fig. 78. Staphylococcus, pure culture and colonies

On meat-peptone agar staphylococci produce well defined colonies with smooth edges, measuring from 1-2 to 2.5 mm in diameter (Fig. 78). Under the microscope the course granular nature of the colonies can be seen, the latter are opaque and have a dense centre. Their colour depends on the pigment produced by the organisms. Besides the typical colony types, staphylococci produce R-, G-, and L-forms. Growth of staphylococci in meat-peptone broth produces diffuse opacity throughout the medium and, subsequently, a precipitate. In some cases when there is sufficient aeration, the organisms form a pellicle on the surface of the broth. Staphylococci grow well on potatoes and coagulated serum. After 24-48 hours incubation there is usually abundant growth along the inoculation stab and liquefaction of gelatin media. On the fourth or fifth day the gelatin medium resembles a funnel filled with fluid.

On blood agar pathogenic staphylococci cause haemolysis of the erythrocytes. Rabbit and sheep erythrocytes are the most sensitive to the staphylococcal haemotoxin.

Fermentative properties. Staphylococci produce enzymes which cause the lysis of proteins and sugars. There is no indole production in young cultures. The organisms liquefy gelatin, coagulate milk and occasionally serum, reduce nitrates to nitrites, produce urease, catalase, phosphatase, ammonia, and hydrogen sulphide. They ferment glucose, levulose, maltose, lactose, saccharose, mannitol, and glycerin, with acid formation.

Toxin production. Pathogenic staphylococci produce an exotoxin which is characterized by lethal, haemolytic, and necrotic activity. Filtrates of staphylococcal broth cultures contain an enterotoxin which causes food poisoning on entry into the gastrointestinal tract. Leucocidin, a substance which destroys leucocytes, haematoblasts of the bone marrow and nerve cells, is also produced by the pathogenic staphylococci. The organisms also coagulate blood plasma. The ability to coagulate plasma is a stable property and is used for differentiating pathogenic from nonpathogenic strains. Coagulase is thermoresistant. It can be isolated from staphylococcal broth cultures.

Staphylococci produce fibrinolysin which when added to a blood clot dissolves the latter within 24-48 hours.

The pathogenic staphylococci produce hyaluronidase which breaks down hyaluronic acid, a component of connective tissue.

Coagulase, fibrinolysin, lecithinase, hyaluronidase and phosphatase all belong to the group of enzymes possessing destructive properties. Lecithinase destroys the lecithin protective membranes of the colloidal particles of a substance found in human, sheep, and rabbit erythrocytes. An anticoagulant which inhibits blood coagulation has also been derived from the staphylococcal cultures. This staphylococcal anticoagulant is produced in exudates of inflamed tissues, occurring during staphylococcal infections. Haemagglutinins which cause the agglutination of rabbit erythrocytes have also been found in staphylococcal culture filtrates. Virulent staphylococci inhibit the phagocytic activity of leucocytes. Substances possessing this property are known as antiphagins. They are thermostable and withstand temperatures up to 80-90°C. Immunization of humans suffering from chronic staphylococcal diseases with antiphagins promotes the formation of agglutinins and opsonins in the blood, which are specific for the given staphylococcal strain, and also precipitins which neutralize the antiphagins.

Due to the variety of properties exhibited by the staphylococcal toxin, certain authors maintain that pathogenic staphylococci produce several toxins. Alpha- and beta-haemolysins have been demonstrated. Many microbiologists believe that the pathogenic staphylococci isolated from sick humans produce alpha-haemolysins, while the organisms pathogenic for animals (e.g., responsible for mastitis in cows) more often produce beta-haemolysin.

Gamma- and delta-haemolysins have also been demonstrated, distinct from the alpha- and beta-fractions. Other toxins which have been demonstrated are the erythrogenic toxin which causes a disease with a similar clinical symptoms to scarlet fever, and a neurotoxin which is poisonous to nerve cells, etc.

However, other authors assume that in spite of its various manifestations, the staphylococcal toxin is chemically homogeneous. This problem is still debatable.

Staphylococcal toxin, inactivated by treatment with 0.3-0.5 per cent formalin at 37°C for 7-28 days, and injected parenterally to humans and animals, stimulates the production of a specific antitoxin capable of reacting with the toxin.

Antigenic structure. Polysaccharides A and B have been obtained from a staphylococcal suspension by treating the latter alternately with acid and alkali and removing the proteins with trichloroacetic acid.

Polysaccharide A was extracted from pathogenic strains isolated from patients with septicaemia, furunculosis, osteomyelitis, and acute conjunctivitis, etc. Polysaccharide B is found in avirulent, nonpathogenic strains. Polysaccharides A and B differ not only in their serological reactions but also in their chemical structures.

Antigen C, containing a specific polysaccharide, has been recently isolated. Staphylococcal polysaccharides demonstrate a marked type specificity. Even in a 1:1,000,000 dilution they give a distinct precipitin reaction. The protein antigen is common to all species and types of staphylococci.

Three types (I, II, III) of staphylococci have been revealed by the agglutination test and precipitin reaction. However, quite a number of cultures are unsuitable for serological typing. Recent studies have revealed fifteen type-specific staphylococcal antigens (a, b, c, etc.)

Classification. The following varieties of staphylococci can be distinguished according to their pigmentation: (a) the golden staphylococcus, *Staphylococcus aureus*; (b) the white staphylococcus, *Staphylococcus albus* (F. Rosenbach, 1884); (c) the lemon-yellow staphylococcus, *Staphylococcus citreus* (Passet, 1885).

But such classification is biased since it takes into account only a single property of the organisms, that of pigment formation which does not always coincide with pathogenicity.

According to the degree of pathogenicity, H. Gross divides the staphylococci into the following groups.

(1) Pathogenic staphylococci which cause the haemolysis of erythrocytes on blood agar (containing a 5 per cent suspension of rabbit or sheep erythrocytes). When inoculated into citrated rabbit blood plasma, they coagulate the latter within 1-2 hours. The cultures cause necrosis of rabbit's skin following intracutaneous injection.

2) Conditionally pathogenic staphylococci which cause slight haemolysis of rabbit and sheep blood on 5 per cent blood agar. These organisms coagulate citrated plasma in 6 hours or more. When injected intracutaneously they produce negligible inflammation (hyperaemia and infiltration).

3) Saprophytes (living on the skin and in the external environment) which do not cause haemolysis, do not coagulate citrated blood plasma and do not produce lesions in the rabbit when injected intracutaneously.

This classification is also inadequate because the pathogenicity of staphylococci depends not only on their biological properties but also on the condition, resistance, and reactivity of the macroorganism.

D. Bergey describes two species in the genus *Staphylococcus* (family *Coccaceae*): *Staphylococcus aureus* and *Staphylococcus epidermidis* (the agent responsible for skin and mucous membrane lesions of human beings and animals). *Staphylococcus epidermidis* has a diameter of 0.5-0.6μ, and occurs singly, in pairs or in irregular clusters. Unlike the golden staphylococcus, it does not ferment mannitol (Table 14).

Table 14

Differentiation of Pathogenic Staphylococci

	Staph. aureus	*Staph. epidermidis*
Mannitol fermentation	+	∓
Production of:		
pigment	Golden, lemon-yellow, off-white	Off-white
alpha-haemolysin	+	∓
hyaluronidase	+	∓
fibrinolysin	+	—

where: + indicates that the organism ferments mannitol, produces haemolysin, hyaluronidase, fibrinolysin;
∓ the organism does not ferment or rarely ferments mannitol, rarely produces alpha-haemolysin, hyaluronidase;
— the organism does not produce fibrinolysin.

On the basis of their susceptibility to bacteriophage staphylococci are classified into about forty types. Certain strains of the family *Micrococcaceae* are strict anaerobes. *Peptoccoccus niger*, *Peptococcus anaerobius*, *Peptococcus asaccharolyticus* and others are usually conditionally pathogenic for human beings. They live in the mouth mucosa, in the intestines, urogenital tract, and in other parts of the human body. In weakened individuals and in people suffering from chronic diseases the anaerobic micrococci may give rise to various diseases and complications.

Resistance. Staphylococci are characterized by a relatively strong resistance to desiccation, freezing, sunlight, and chemical substances. After desiccation they can survive for more than 6 months. Repeated freezing and thawing do not kill the organisms. They survive for many hours under direct sunlight. Staphylococci maintain their viability for more than 1 hour at 70°C. At a temperature of 80°C they are destroyed within 10-60 minutes and at boiling point, they instantly perish. A 5 per cent phenol solution kills

the organisms in 15-30 minutes. Staphylococci are very sensitive to certain aniline dyes, particularly to brilliant green which is used for treating pyogenic skin diseases caused by these organisms.

Pathogenicity for animals. Horses, cattle, sheep, goats, pigs, and, among laboratory animals, rabbits, white mice, and kittens are susceptible to pathogenic staphylococci.

An intracutaneous injection of a culture of pathogenic staphylococci produces inflammation and subsequent necrosis in the skin of the rabbit. An intravenous injection of a staphylococcal culture filtrate causes a condition similar to acute poisoning in rabbits, which is characterized by motoric excitation, respiratory disorders, convulsions, paralysis of the hind extremities, and sometimes, by diarrhoea and urine discharge. After complete fatigue the animal shortly dies.

Pathogenic staphylococci or their toxin will cause vomiting, diarrhoea, and weakness in kittens if introduced per os or intraperitoneally. Functional disorders of the digestive tract arise owing to the effect of the enterotoxin which is distinguished from the other fractions of the staphylococcal toxin by its thermoresistance. It withstands a temperature of 100°C for 30 minutes. The most reliable test for the presence of enterotoxin is an intravenous injection to adult cats.

Pathogenesis and diseases in man. Staphylococci enter the body through the skin and mucous membranes. When they overcome the lymphatic barrier and penetrate the blood, staphylococcal septicaemia sets in. Both the exotoxins and the bacterial cells play an important role in pathogenesis of diseases caused by these organisms. Consequently, staphylococcal diseases should be regarded as toxinfections.

The development of staphylococcal diseases is also influenced by the resulting allergy which in many cases is the cause of severe clinical forms of staphylococcal infections which do not succumb to treatment.

Pathogenic staphylococci are responsible for a number of local lesions in humans: hidradenitis, abscess, paronychia blepharitis, furuncle, carbuncle, periostitis, osteomyelitis, folliculitis, sycosis, dermatitis, eczema, chronic pyodermia, peritonitis, meningitis, appendicitis, and cholecystitis.

Diabetes mellitus, avitaminosis, alimentary dystrophy, excess perspiration, minor occupational skin abrasions, as well as skin irritation caused by chemical substances, are some examples of the conditions conducive to the formation of pyogenic lesions of the skin and furunculosis.

In some cases staphylococci may give rise to secondary infection in individuals suffering from smallpox, influenza, and wounds, as well as postoperative suppurations. Staphylococcal sepsis and staphylococcal pneumonia in children are particularly severe diseases. Ingestion of foodstuffs (cheese, curds, milk, rich cakes and pastry,

ice cream, etc.) contaminated with pathogenic staphylococci may result in food poisoning.

Staphylococci play an essential part in mixed infections, and are found together with streptococci in cases of wound infections, diphtheria, tuberculosis, actinomycosis, and angina.

Immunity. The tendency to run a chronic flaccid course or relapse is regarded as a characteristic symptom of staphylococcal infections. This peculiarity gives a basis for concluding that postinfectional immunity following staphylococcal diseases is of low grade and short duration.

Immunity acquired after staphylococcal diseases is due to phagocytosis and the presence of antibodies (antitoxins, precipitins, opsonins, and agglutinins).

The inflammation restricts the staphylococci to the site of penetration and obstructs their spreading throughout the body. At the centre of inflammation the organisms undergo phagocytosis. Neutralization of the staphylococcal toxin by the antitoxin is an important stage of the immunity complex. As a result of capillary permeability, the antitoxin penetrates from the blood into the inflammation zone and renders harmless the toxin produced by staphylococci. Thus, the phagocytic and humoral factors act together and supplement each other.

Laboratory diagnosis. Test material may be obtained from pus, mucous membrane discharge, sputum, urine, blood, foodstuffs (cheese, curds, milk, pastry, cakes, cream, etc.), vomit, lavage fluids, and faeces.

The material is examined for the presence of pathogenic staphylococci. Special rules are observed when collecting the material since nonpathogenic strains are widespread in nature.

Laboratory studies comprise the determination of the main properties of the isolated staphylococci (i.e., their morphologic, cultural and biochemical characteristics), as well as their virulence. For this purpose the following procedures are carried out. Smears are made from pus and stained by the Gram method. Pus is inoculated onto blood agar and meat-peptone agar containing crystal violet. In cases of septicaemia blood is inoculated into glucose broth. The isolated pure culture is tested for its haemolytic (by inoculation onto blood agar plates), plasmacoagulative (by inoculation into citrated rabbit plasma), and hyaluronidase activities. Virulence is determined on rabbits by intracutaneous injection of 400 million microbial cells. Necrosis develops at the site of injection within 24-48 hours. Pigment production of the isolated culture is also taken into account. For revealing sources of infection, particularly food poisoning and outbreaks of sepsis in maternity hospitals, serological typing and phage typing are carried out. To ensure effective therapy the isolated cultures are examined for sensitivity to antibiotics.

In cases of food poisoning presence of the enterotoxin in the isolated staphylococcal culture is tested for by intravenous injection of the culture filtrate to adult cats. In cases when intoxication is due to ingestion of the milk of a cow suffering from mastitis, the culture grown on starch medium is tested directly for toxin production as a means of detecting staphylococci of animal origin.

Treatment. Staphylococcal diseases are treated with antibiotics (penicillin, phenoxymethyl penicillin, chlortetracycline, tetracycline, gramicidin, microcide, sanasine, etc.) and sulphonamides (norsulphazol, sulphazine, etc.).

In cases of chronic staphylococcal lesions specific therapy is recommended: autovaccines, staphylococcal anatoxin, staphylococcal antiphagin, antitoxic serum, and diphage containing staphylococcal and streptococcal phages.

Staphylococci produce strains resistant to sulphonamides, antibiotics, and bacteriophage, which advances the wide distribution of staphylococcal infection. This variability is of particular importance in the therapy of staphylococcal pyogenic diseases. The medical services produce semisynthetic preparations of penicillin and tetracycline which are effectively used for the treatment of these diseases.

Prophylaxis. The general precautionary measures include: hygiene in working and everyday-life conditions, treatment of vitamin deficiency, prevention of traumatism and excess perspiration, observance of rules of hygiene in maternity hospitals, surgical departments, children's institutions, industrial plants and enterprises, particularly canneries, observance of personal hygiene and frequent washing of hands in warm water with soap.

Routine disinfection of hospital premises (surgical departments, maternity wards) and bacteriological examination of the personnel for carriers of pathogenic staphylococci resistant to antibiotics are also necessary.

Protective liniments and ointments are applied for the prevention of pyodermia at industrial enterprises. Besides the application of tincture of iodine and alcoholic solutions of aniline dyes for treatment of micro-injuries, wide use is made of liquid preparations which dry in 1-2 minutes forming an elastic film which protects the wound from contamination and infection. Specific prophylactic immunization with staphylococcal anatoxin may be recommended in certain cases for individuals subject to frequent injuries.

STREPTOCOCCI

The pathogenic streptococcus (*Streptococcus pyogenes*) was discovered by T. Billroth (1874) in tissues of patients with erysipelas and wound infections and by L. Pasteur et al. (1880) in patients

with sepsis. A. Ogston described the organisms in studies of suppurative lesions (1881). A pure culture of the organism was isolated by F. Fehleisen (1883) from a patient with erysipelas and by F. Rosenbach (1884) from pus. Streptococci belong to the family *Lactobacillaceae*.

Morphology. Streptococci are spherical in shape, 0.6 to 1 μ in diameter, and form chains (Fig. 79*a*). They are nonmotile (although

Fig. 79. Streptococci

a—arrangement of cells in pus; *b*—colonies of haemolytic streptococcus on blood agar; *c*—smear from a broth culture

motile forms are encountered), do not form spores and are Gram-positive. Some strains are capsulated. In smears from cultures grown on solid media the streptococci are usually present in pairs or in short chains, while in smears from broth cultures they form long chains or clusters (Fig. 79*c*).

Cultivation. Streptococci are aerobic and facultatively aerobic, and there are also anaerobic species. The optimal temperature for growth is 37°C, and no growth occurs beyond the limits of 20-40°C (for enterococci the limits are 10-45°C).

The organisms show poor growth on ordinary meat-peptone agar, and grow well on sugar, blood, serum and ascitic agar and broth, when the pH of the media is 7.2-7.6. On solid media they produce small (0.5-1 mm in diameter), translucent, grey or greyish-white, and granular colonies with poorly defined margins. Some streptococcal strains cause haemolysis on blood agar (Fig. 79*b*), others produce a green coloration surrounding the colony, 1-2 mm in diameter, as a result of conversion of haemoglobin into methaemoglobin, while others do not cause any change in the erythrocytes. On sugar broth medium growth is in the form of fine-granular precipitates on the walls and at the bottom of the tube and only rarely does the broth become turbid.

Fermentative properties. Pathogenic streptococci are nonproteolytic, do not liquefy gelatin, and do not reduce nitrates to nitrites. They coagulate milk, dissolve fibrin, ferment glucose, maltose, lactose, saccharose, mannitol (not always constantly), and break down salicin and trehalose, with acid formation.

Toxin production. Pathogenic streptococci produce exotoxins with various activities:

(1) haemolysin (haemotoxin, streptolysin) which loses its activity after 30 minutes at a temperature of 55°C; disintegrates erythrocytes; produces haemoglobinaemia and haematuria in rabbits following intravenous injection;

(2) leukocidin which is destructive to leucocytes; occurs in highly virulent strains and is rendered harmless by a temperature of 70°C;

(3) necrotoxin which produces necrosis in rabbits when injected intracutaneously; it also causes necrosis in other tissues, particularly in the hepatic cells;

(4) lethal toxin which rapidly kills rabbits and white mice when injected intravenously;

(5) erythrogenic toxin produces inflammation in humans who have no antitoxins in their blood.

Other substances produced by the pathogenic streptococci are harmful enzymes. They include hyaluronidase (which facilitates the spread of the organisms throughout the tissues and organs of the affected animal), fibrinolysin, desoxyribonuclease, ribonuclease, proteinase, amylase, lipase, and diphosphopyridine nucleotidase. Streptococcal phages possess transduction properties and may be responsible for increased toxigenicity of *C. diphtheriae* and increased virulence of other bacteria occurring in association with streptococci.

Endotoxins, characterized by their thermoresistance and specific activity, are responsible for the pathogenic properties of streptococci together with the exotoxins and aggressive enzymes.

Antigenic structure. The study of the antigenic structure of streptococci is based on serologic examinations. F. Griffith used the agglutination test, while R. Lancefield employed the precipitin reaction with an extract of a broth culture precipitate.

Four antigenic fractions were recovered from streptococci: the type-specific protein (M- and T-substances); group-specific polysaccharide (C-substance), and nucleoprotein (P-substance). The M-substance is a protein which confers type specificity, virulence, and immunogenicity. The T-substance contains O-, K-, and L-antigens. The C-substance is a polysaccharide common to the whole group of haemolytic streptococci. The P-substance belongs to the nucleoprotein fraction, being nonspecific for haemolytic streptococci; it contains nucleoproteids common to other groups of streptococci, as well as to pneumococci and staphylococci.

Group A and, partly, group C and G streptococci possess extra-

cellular antigens: streptolysin O, a protein which causes erythrocyte haemolysis, and streptolysin S, a lipoprotein complex possessing erythrocytolytic activity.

The streptococci were divided into 17 species, using the precipitin technique which is based on the detection of group-specific carbohydrates. These species are responsible for various human and animal diseases (Table 15).

Table 15

Diseases Caused by Streptococci of Various Groups

Group	Disease Caused	Habitat
A	Majority of human streptococcal diseases	Man
B	Mastitis in cows. Postnatal infections in humans and sepsis in newly born animals	Cow Human genital tract
C	Diseases in various animals. Mild respiratory infections in humans	Various animals Upper respiratory tract of humans
D	Infections of urogenital tract in humans. Endocarditis. Wound infections	Milk products Intestines of humans and animals
E	Diseases in pigs and cows	Pigs, cows
F	Respiratory infections in humans. Endocarditis	Upper respiratory tract of humans
G	Mild respiratory infections in humans. Genital tract infections in dogs	Upper respiratory tract of humans. Dogs
H	Endocarditis	Upper respiratory tract of humans
K	Endocarditis	Upper respiratory tract of humans
L	Genital tract infections in dogs	Dogs
M	Genital tract infections in dogs	Dogs
N	Endocarditis	Dogs
O	Endocarditis	Milk products
P	Not known	Chickens. Pigs
Q	Not known	Human intestines
R	Not known	Human intestines
S	Not known	Human intestines

The haemolytic streptococci, recovered from sick human beings, were subdivided by F. Griffith into 51 serologic types. He attributed 47 types to group A, types 7, 20, and 21 to group C, and type 16 to group G.

Classification. Previously streptococci were classified (H. Schotmüller, M. Brown, et al.) on the basis of their behaviour on blood agar. According to this classification, streptococci are subdivided into the following species: (1) *Streptococcus haemolyticus* (β)—

haemolytic streptococcus; (2) *Streptococcus viridans* (α)—streptococcus which produces greenish haloes on blood agar; (3) *Streptococcus viridans* (α_1)—produces a less distinct and opaque zone of haemolysis as compared to α-haemolysis; (4) *Streptococcus anhaemolyticus* (γ)—nonhaemolytic streptococcus.

D. Bergey subdivides the streptococci into three groups:

(1) pyogenic streptococci (*Str. pyogenes, Str. equisimilis, Str. zooepidemicus, Str. equi, Str. dysgalacticae, Str. sanquis, Str. anginosus, Str. agalactiae*);

(2) viridans streptococci (*Str. acidominimus, Str. salivarius, Str. mitis, Str. bovis, Str. equinus, Str. thermophilus, Str. uberis, Str. faecalis, Str. durans*);

(3) lactic streptococci (*Str. lactis, Str. cremoris*).

The current classification is based on the grounds of detection of group-specific polysaccharide antigens. According to the presence of these antigens all streptococci are subdivided into groups, among which groups A, B, C, and D are of the most practical importance.

Group A is most important in relation to infectious pathology. It comprises 47 strains of haemolytic streptococci which can be isolated from sick people and from the nasopharynx of healthy individuals. These streptococcal strains cause erythrocyte haemolysis, produce a soluble toxin, and do not grow on 40 per cent bile broth and bile-blood agar. They usually ferment lactose and salicin, and always trehalose. The strains often ferment mannitol but rarely raffinose and inulin. They do not ferment sorbitol or glycerin. The organisms are found in patients with tonsillitis, scarlet fever, erysipelas, and other inflammatory processes.

The enterococci (*Streptococcus faecalis, Str. faecium, Str. durans*) are pleomorphic oval cells which occur in pairs or in short chains. Some are oval or spear-shaped in form, similar to that of the pneumococci. The organisms are from 0.5 to 1 μ in diameter. Haemolytic types (*Streptococcus faecalis, var. zymogenes*) and organisms liquefying gelatin (*Streptococcus faecalis, var. liquefaciens*) are found. According to their antigenic structure, enterococci are divided into six O-groups among which there are strains with K-antigens (capsular antigens). Some enterococcal and lactic streptococci possess identical antigens. On solid media enterococcal growths form a thin pellicle with smooth edges. On sugar broth they produce turbidity and precipitate. Certain enterococci are highly motile. Some of the strains produce a yellow pigment, and the pathogenic enterococci produce fibrinolysin. The organisms grow at temperatures ranging from 10 to 45°C. They are resistant to high temperature (e.g., withstand exposure to 60°C for half an hour). Enterococci can be grown in broth containing 6.5 per cent common salt at pH 9.6 and on blood agar containing 40 per cent bile or an equivalent amount of bile salts. They ferment glucose, maltose lactose, mannitol, trehalose, salicin, and inulin, with acid for-

mation. They reduce and coagulate litmus milk in the presence of 0.1 per cent methylene blue. Enterococci differ from other streptococci in their ability to grow over a wide range of temperatures (10-45°C) and in a medium of pH 9.6, in their resistance to high concentrations of salt and to penicillin (a number of strains show growth in media containing 0.5-1 U of antibiotic per 1 ml of media). All enterococci decarboxylate tyrosine.

Enterococci inhabit the small and large intestines of man and warm-blooded animals. The organisms possess properties antagonistic to dysenteria, enteric fever, and paratyphoid bacteria, and to the coli bacillus. In the child's intestines the enterococci are more numerous than the *Escherichia coli*. In lesions of the duodenum, gall bladder, and urinary tract enterococci are found as a result of dysbacteriosis. Isolation of enterococci serves as a criterion of contamination of water, sewage, and foodstuffs with faeces (see p. 109).

Of special interest are the alpha-streptococci (the viridans group). They are responsible for haemometamorphosis on blood agar (greenish discoloration of the media) and produce no soluble haemolysin. The organisms of this group usually ferment raffinose and do not ferment mannitol. They are always isolated from the mouth and throat of healthy people and have low virulence for humans and animals. The viridans streptococci are found in pyogenic and inflammatory lesions of the teeth and gums and are responsible for subacute endocarditis.

Anaerobic streptococci (*Peptostreptococcus putridus, Peptostreptococcus anaerobius*, and others) are the cause of severe puerperal septic infections (puerperal sepsis). They are isolated from pyogenic and gangrenous lesions which have a putrefactive odour.

Resistance. Streptococci live for a long time at low temperatures, are resistant to desiccation, and survive for many months in pus and sputum. When exposed to a temperature of 70°C, they are destroyed within one hour. A 3-5 per cent phenol solution kills the organisms within 15 minutes.

Pathogenicity for animals. Cattle, horses, and, among laboratory animals, rabbits and white mice are susceptible to the pathogenic streptococci.

The virulence of streptococci is tested on rabbits. The animals are infected by rubbing a culture suspension into a scratch made on the skin of the ear or on the back. This results in a local inflammation with the appearance of hyperaemia and swelling. An intravenous injection of pathogenic streptococci causes septicaemia or selectively affects the lungs, liver, kidneys, or joints.

Pathogenesis and diseases in man. The pathogenesis of streptococcal infections is brought about by the effect of the exotoxin and the bacterial cells. The reactivity of the infected body and its previous resistance play an important part in the origin and development of streptococcal diseases. Such diseases as endocarditis, polyar-

thritis, highmoritis, chronic tonsillitis, and erysipelas are associated with abnormal body reactivity, hyperergia. This condition may persist for a long period of time and serve as the main factor for the development of chronic streptococcal diseases.

With an exogenous mode of infection streptococci invade the human body from without (from sick people and animals, various contaminated objects and foodstuffs). They gain access through injured skin and mucous membranes or enter the intestines with the food. Streptococci are mainly spread by the air droplet route. When the natural body resistance is weakened, conditionally pathogenic streptococci normally present in the human body become pathogenic. Penetrating deep into the tissues they produce local pyogenic inflammations, such as streptoderma, abscesses, phlegmons, lymphadenitis, lymphangitis, cystitis, pyelitis, cholecystitis, and peritonitis. Erysipelas (inflammation of the superficial lymphatic vessels) and tonsillitis (inflammation of the pharyngeal and tonsillar mucosa) are among the diseases caused by streptococci. Invading the blood, streptococci produce a serious septic condition. They are more commonly the cause of puerperal sepsis than other bacteria. The importance of streptococci in scarlet fever is discussed below.

Streptococci may cause secondary infections in patients with diphtheria, smallpox, whooping cough, measles, and other diseases. Chronic tonsillitis is attributed to the viridans streptococci and adenoviruses.

Contamination of wounds with streptococci during war results in wound suppurations, abscess formation, phlegmons, and traumatic sepsis.

Immunity. Immunity acquired after streptococcal infections is of a low grade and short duration. Relapses of erysipelas, freguent tonsillitis, dermatitis, periostitis, and osteomyelitis occur as a result of sensibilization of the body. This is attributed to low immunogenic activity and high allergen content of the streptococci, as well as to the presence of numerous types of the organisms against which no cross immunity is produced.

Immunity following streptococcal infections is of an anti-infectious nature. It is associated with antitoxic and antibacterial factors. The antitoxins neutralize the streptococcal toxin and together with the opsonins facilitate phagocytosis.

Laboratory diagnosis. Test material is obtained from the pus of wounds, inflammatory exudate, tonsillar swabs, blood, urine, and foodstuffs. Procedures are the same as for staphylococcal infections. Tests include microscopy of pus smears, inoculation of test material onto blood agar plates, isolation of the pure culture and its identification. Blood is sown on sugar broth if sepsis is suspected. Virulence is tested on rabbits by an intracutaneous injec-

tion of 200-400 million microbial cells. Toxicity is determined by injecting them intracutaneously with broth culture filtrate.

The group and type of the isolated streptococcus and its resistance to the medicaments used are also determined. In endocarditis there are very few organisms present in the blood in which they appear periodically. For this reason blood in large volumes (20-50 ml) is inoculated into vials containing sugar broth. If possible, the blood should be collected while the patient has a high temperature. In patients with chronic sepsis an examination of the centrifuged urine precipitate and isolation of the organism in pure culture are recommended.

Treatment. Usually penicillin is used. For penicillin-resistant strains, streptomycin, tetracycline, and erythromycin are required. Vaccine therapy (autovaccines and polyvalent vaccines) and phage therapy are recommended in chronic conditions.

In some countries diseases caused by beta-haemolytic streptococci of groups A, C, G, and H and by alpha-streptococci (endocarditis) are treated with anti-infectious (antitoxic and antibacterial) streptococcal sera together with antibiotics and sulphonamides.

Prophylaxis. Streptococcal infections are prevented by the practice of general hygienic measures at factories, children's institutions, maternity hospitals, and surgical departments, in food production, agricultural work, and everyday life.

Penicillin has been widely used by surgeons, gynaecologists, otorhinolaryngologists, and other specialists prior to, during and after operations, for prevention of suppurations.

Maintaining appropriate sanitary levels of living and working conditions, raising the cultural level of the population, and checking personal hygiene are of great importance.

There is no specific prophylaxis for streptococcal infections on account of the low immunogenic activity of streptococci and the large number of type varieties which do not produce cross immunity.

ROLE OF STREPTOCOCCUS IN THE AETIOLOGY OF SCARLET FEVER

Scarlet fever has long been known as a widespread disease but at the present time its aetiology has not yet been ascertained. Four different theories were proposed: streptococcal, allergic, viral, and combined (viral-streptococcal). Most scientists and medical practitioners favoured the streptococcal theory.

G. Gabrichevsky in 1902 was the first to point out the aetiological role of the haemolytic streptococcus in scarlet fever. Usually he recovered the organisms from the pharynx of scarlet fever patients and from blood contained in the heart of those that had died of the disease. In 1907 he prepared vaccine from killed scarlet fever haemo-

lytic streptococcal cultures. This vaccine was widely used for human vaccination.

In 1905 I. Savchenko, cultivating scarlet fever streptococci, obtained the toxin and used it for hyperimmunization of horses. The antitoxic antiscarlatinal serum was effectively used for treating people suffering from scarlet fever.

Data presented by Gabrichevsky and Savchenko concerning the streptococcal theory were confirmed by studies carried out in 1923-24 by G. Dick and G. Dick and by many other scientists.

The streptococcal aetiology of scarlet fever is supported by the following arguments: (1) all people suffering from scarlet fever are found to harbour in their throats haemolytic streptococci which are agglutinated by the sera of convalescents; (2) a subcutaneous injection of the scarlet fever toxin into susceptible people (volunteers) in some cases is followed by the appearance of a characteristic skin rash, vomiting, fever, tonsillitis, and other scarlatinal symptoms; (3) an intracutaneous injection of the toxin into susceptible children produces a local erythematous and oedematous reaction; the toxin produces no reaction in children who had previously suffered from scarlet fever and were immune to the disease; (4) if 0.1 ml of antitoxic antistreptococcal serum or convalescent serum is introduced into the skin of a scarlet fever patient in the area of the rash, the latter turns pale (is "extinguished"); (5) hyperimmunization of animals with the scarlet fever toxin leads to the production of antitoxins, and a neutralization reaction takes place between the toxin and antitoxins; (6) therapy with antitoxic sera and prophylaxis with combined vaccines consisting of the toxin and haemolytic streptococcal cells result in the appearance of less severe cases and decrease in morbidity and mortality.

At present many investigators accept the streptococcal theory in scarlet fever aetiology. In postwar years this theory has been confirmed by a number of investigations. Arguments against the streptococcal theory are as follows: (1) people inoculated with scarlet fever streptococci or their toxins do not always display the characteristic symptoms of the disease, e. g., there is no peeling, only rarely are there instances of tonsillitis, and phlegmon, sepsis, and erysipelas occasionally develop; (2) in severe hypertoxic forms the antitoxic serum has little effect, while the serum of convalescents gives better results; (3) the skin toxin test (Dick test) sometimes gives a negative reaction with susceptible children and produces a positive reaction with those who are immune; (4) immunity acquired after scarlet fever is very stable and of long duration, while that acquired after other streptococcal diseases is unstable, of short duration, and is frequently accompanied by an increased susceptibility to streptococci.

Some authors believe scarlet fever to be an allergic reaction caused by reinfection of children with haemolytic streptococci. The scarlet

fever streptococcus has been shown to possess two toxic fractions. One of them is thermolabile and produces a skin reaction in susceptible individuals (erythrogenic exotoxin), and the other is thermostable and possesses allergenic properties. The above point of view is not generally accepted since it contradicts the established infectiousness of scarlet fever.

Some investigators attributed a particular role in the aetiology of scarlet fever to a virus (I. Kantakusino, S. Zlatogorov, and others). According to M. Morozov, the scarlet fever virus possesses epitheliotropic properties and is about 150-200 mμ in size. It is visible under an ordinary light microscope with a magnification of 1,500-2,000. The virus can be grown on tissue cultures.

S. Zlatogorov believed that the pathogenesis of scarlet fever depends on the joint action of streptococci of the scarlet fever type and a virus which activates the streptococcus and endows it with special properties. H. Tsishinsky, G. Vildfura, and others are of the opinion that scarlet fever is initially of viral origin but later becomes a bacterial infection, this latter stage developing as a complication. It is probable that scarlet fever is an infection resulting from the association of the haemolytic streptococcus and viruses. This is similar to certain cases of tonsillitis in the aetiology of which the role of the viridans streptococcus and certain viruses has been established.

Scarlet fever streptococci do not differ from the haemolytic streptococci of the Lancefield A group in their morphologic, cultural, fermentative, or other properties. Serological tests show that the I and IV types of Griffith streptococcus are most frequently isolated.

Pathogenesis and disease in man. People become infected by the air droplet route. Sick people, convalescents, and carriers of the causative agent of scarlet fever are all sources of infection. The disease is most commonly encountered in children from 1 to 8 years of age. The bacterial cells themselves together with their exotoxins play an important role in the pathogenesis of scarlet fever.

Allergy to scarlet fever is demonstrated by the Aristovsky-Fanconi phenomenon. An intracutaneous injection of killed streptococci into individuals recovering from scarlet fever produces erythema, swelling, and pain at the site of injection.

The causative agent sometimes enters the body through wounds on the skin and mucous membranes of the genitalia. This form of scarlet fever is known as extrabuccal or extrapharyngeal (traumatic, combustion, surgical, and puerperal). Certain objects (e.g., utensils, toys, books, etc.) as well as foodstuffs (e.g., milk), contaminated by adult carriers, may also be sources of infection. Of great importance in the epidemiology of scarlet fever are the patients with atypical, unrecognizable forms of the disease. In its initial stage scarlet fever is chiefly characterized by intoxication, while in the second stage it is accompanied by septic and allergic conditions.

Immunity. Scarlet fever produces a relatively stable immunity. Reinfections are very rare. They have increased in number in the last years as a result of wide use of antibiotics which reduce the immunogenic activity of the pathogen and its toxin.

Data concerning the correlation between a positive Dick test and susceptibility to scarlet fever provide evidence of the antitoxic nature of immunity acquired after scarlet fever. Children from 1 to 5 years are most susceptible.

According to data of A. Tsinger, among healthy children, 48 per cent up to the age of 6 months, 64-71 per cent between the ages of 6 months and 3 years, 46-56 per cent between the ages of 3 and 5 years, and 24-37 per cent between the ages of 5-20 years give a positive Dick test.

Laboratory diagnosis. Scarlet fever is recognized mainly by its clinical course and on epidemiological grounds. Laboratory diagnosis for the detection of haemolytic streptococci and their typing is employed only in certain cases. This method is of no practical value since haemolytic streptococci are often isolated from people with various diseases and frequently from healthy individuals.

The phenomenon of rash "extinguishment" is employed as an auxiliary diagnostic method. For this practice, 0.1 ml of antitoxic antiscarlatinal serum or a convalescent serum is injected intracutaneously into the patient. If he has scarlet fever, the rash at the site of injection will disappear within 12-20 hours and the skin will turn pale.

Certain physicians apply the Dick test with the thermolabile fraction of the toxin. The diagnosis of scarlet fever is verified to a certain extent if on a second injection of the toxin a positive Dick test reverts to a negative reaction.

Scarlet fever may also be diagnosed by detecting precipitins in the urine (urine precipitation test). A layer of type-specific streptococcal sera or convalescent serum is transferred onto freshly filtered urine of patients in the first days of the disease. The appearance of a greyish-white ring at the interface of the two fluids designates a positive reaction. However, the specificity of the test is only relative.

Treatment. Scarlet fever patients are treated with penicillin, tetracycline, levomycetin, sulphonamides (norsulphazol, etc.), and gamma-globulin from human blood. Antitoxic antiscarlatinal serum (20,000-60,000 U) is administered in toxic and toxico-septic forms of the disease. The wide use of antibiotics in postwar years has led to a significant decrease in the morbidity and mortality rate of scarlet fever and to a milder course of the disease. This fact also confirms the definite role played by haemolytic streptococci in the aetiology and pathogenesis of scarlet fever, since it is known that these organisms are extremely sensitive to penicillin and other antibiotics.

Prophylaxis consists of early diagnosis, isolation of patients and hospitalization in the presence of epidemiological and clinical indications. Extremely hygienic cleaning and ventilation and observance of correct hospital regimen are also necessary. If cases of scarlet fever occur in children's institutions, the children concerned must be isolated. Debilitated children who have been in contact with scarlet fever patients must be injected with 1.5-3 ml of human serum gamma-globulin.

No specific prophylaxis of wide scope has been carried out among children in recent years since no exact information on scarlet fever aetiology is available. An adsorbed scarlet fever toxin which is responsible for the production of antitoxic immunity in the body is undergoing trial at present.

A compound diphtheria-whooping cough-scarlet fever vaccine is also prepared. One millilitre of the vaccine contains 60 Units of pure diphtheria anatoxin, 40,000 million pertussis bacterial cells, and a highly purified scarlet fever (erythrogenic) toxin. The compound vaccine is used for immunization of 5-6-month-old children who have not suffered from scarlet fever and whooping cough and for whom vaccination against diphtheria is compulsory.

ROLE OF STREPTOCOCCUS IN THE AETIOLOGY OF RHEUMATIC FEVER

The majority of authors maintain that rheumatic fever develops as a result of the body becoming infected by group A beta-haemolytic streptococci. Acute or chronic tonsillitis and pharyngitis produce a change in the immunological reactivity of the body and this gives rise to characteristic clinical symptoms and a pathological reaction.

The prevalence of rheumatic fever depends on the time of the year. The highest number of cases occurs in October-November and March-April. Acute and chronic tonsillitis, pharyngitis, and catarrh are also most prevalent in these months.

Pathogenesis of rheumatic fever. The allergic reaction produced in the body as a result of re-invasion by antigens (streptococcal exo- and endotoxins, autoantigens, and complexes consisting of streptococcal toxins and components of tissue and blood proteins of sick people) is an important factor in the pathogenesis of the disease. It is known that blood of individuals who have suffered from a streptococcal infection contains antibodies against beta-haemolytic streptococci. In 1-3 per cent of these cases the formation of antibodies does not produce immunity, and a secondary invasion of the body by specific and nospecific antigens leads to the development of hyperergia. Experiments have shown that streptococci bring about the formation of autoantigens which cause the production of autoantibodies in the body. These autoantibodies are responsible for lesions in certain tissues and organs.

Studies of high-molecular gamma-globulins and their complexes in rheumatic fever have shown that the normal human gamma-globulin contains two fractions (7S and 19S) which differ in their precipitate constant. The majority of the common antibodies are associated with the 7S gamma-globulin fraction, while isoagglutinins, Rh-agglutinins, and complement-fixing antibodies are contained in the 19S fraction. The gamma-globulin fraction, rich in 19S, is found to contain the rheumatoid factor. An interaction has been demonstrated between the rheumatoid factor and the antigen-antibody precipitate, the latter possessing antigenic properties. Thus, in its reaction with the antigen-antibody complex the rheumatoid factor behaves as the complement. Alternatively, the rheumatoid factor may act as an antibody to gamma-globulin or to the antigen-antibody complex, the latter acting as an antigen.

Antigen-antibody reactions result in the injury of the interstitial connective tissue, release of histamine, and inflammation. Disturbances of coordination in the hypophysis-adrenal system are encountered in rheumatic fever. For this reason supporters of the Selye theory consider rheumatic fever to be an adaptational disease. However, the above-mentioned aspects on the mechanism of rheumatic fever can by no means cover all the complex processes involved in the pathogenesis of this disease. Functional disturbances of the central nervous system, various types of higher nervous activity, heredity and other factors are significant in the development of this disease. Certain research workers believe rheumatic fever to be a disease of viral aetiology.

According to its **clinical course**, rheumatic fever is differentiated into active and inactive phases. The active phase is characterized by acute rheumocarditis without valvular defects and relapsing rheumocarditis accompanied by valvular defects, polyarthritis, chorea, pleuritis, peritonitis, nephritis, hepatitis, pneumonia, lesions in the skin and subcutaneous tissue, eyes, and other systems. The inactive phase develops in the form of rheumatic myocardiosclerosis, heart defects, and conditions following extracardial affections.

Three periods can be distinguished during the development of rheumatic fever: (1) period of acute streptococcal infection and initial sensitization; (2) period of hyperergic reactions, resulting from interaction between antigens and antibodies, which are accompanied by primary rheumatic polyarthritis or carditis; (3) period of stable allergic reactivity accompanied by pronounced manifestations of parallergy and autosensitization, profound and stable immunogenic disturbances, and relapses.

Laboratory diagnosis is made on the basis of determination of an increase in antistreptolysin, antifibrinolysin, and antihyaluronidase titres and detection of C-reactive protein.

Treatment of rheumatic patients is accomplished by several measures aimed at desensibilization of the body, abatement of inflam-

matory conditions, recovery of normal body reactivity, condition of the nervous system, and disturbed processes, and control of local infections.

Prophylaxis includes prevention of streptococcal infections, strengthening of general resistance, and creation of favourable conditions for everyday life and work. In addition, all people suffering from rheumatic fever and those susceptible to the disease should be given prophylactic treatment with penicillin and tetracycline preparations in spring and autumn.

PNEUMOCOCCI

Pneumococcus (*Diplococcus pneumoniae*) has been described by R. Koch (1871), E. Klebs (1875), L. Pasteur, S. Chamberlain, and E. Roux (1881). K. Frankel (1885) and A. Weichselbaum (1886) isolated a pure culture of the organism from the sputum of a patient. N. Gamaleya (1888) classified it with the lanceolate streptococcus. Pneumococci belong to the family *Lactobacillaceae*.

Morphology. Pneumococci are lanceolate or slightly elongated cocci measuring 0.5-1.25 μ in diameter. They occur in pairs (Fig. 80) and sometimes form short chains. In the bodies of human beings and animals they are enclosed in capsules (see Fig. 117, 9). The organisms are Gram-positive, nonmotile, and have no spores.

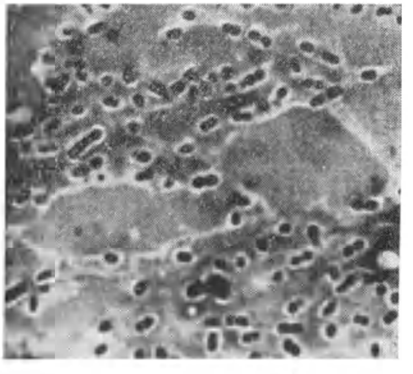


Fig. 80. Pneumococci in patient's sputum

Cultivation. Pneumococci are aerobes or facultative aerobes. The optimum temperature for growth is 37°C, and the growth temperature limits are 28° and 42°C. The organisms show no growth at 25°C. Cultivation on ordinary media is poor. They grow readily on serum or ascitic agar at pH 7.2-7.6 on which they appear as small colonies 1 mm in diameter. After 48 hours the colonies become larger having a central depressed portion and raised edges. On blood agar pneumococci form small, rounded, succulent colonies surrounded by a green zone in the medium. On sugar broth they produce turbidity and a precipitate. The organisms grow well in broth containing 0.2 per cent glucose. On artificial media normally no capsules are formed, but addition of animal protein to a fluid medium enhances the formation of the capsules.

Dissociation of M-type pneumococci to S- and R-types takes place under the influence of factors in the external environment. Streptococcal-like forms of pneumococci appear in the presence of low concentrations of optochin.

Fermentative properties. The pneumococcus does not liquefy gelatin, produces no indole, and does not reduce nitrates to nitrites. It coagulates milk, ferments glucose, maltose, lactose, saccharose, inulin, raffinose, and salicin. Virulent pneumococci are soluble in bile and bile salts. Fermentation of inulin and solubility in bile and bile salts are important points for differentiation between pneumococci and streptococci.

Toxin production. No soluble toxin is produced by pneumococci. The organisms contain an endotoxin and liberate poorly a toxin which is characterized by its haemotoxic and fibrinolytic properties and by its ability to destroy leucocytes.

Pneumococcal virulence is associated with the capsule which contains compounds reducing phagocytosis and the bactericidal properties of the blood. The capsules of virulent pneumococci contain specific components, known as antiphagins (virulins). These substances are not toxic, but when added to nonvirulent pneumococci, render the latter highly resistant to phagocytosis. Antiphagins are thermoresistant and withstand temperatures of 80-100°C.

Antigenic structure and classification. Polysaccharide complexes (specific polysaccharides) are present in the pneumococcal capsules. They are responsible for type specificity and virulence.

Protein antigens are common to all types. They are found in the cytoplasm, and are not responsible for specificity or virulence.

Pneumococci are characterized by numerous serological varieties. There are 80 types which are agglutinated only by the serum of the corresponding type. Types I, II, and III are virulent for human beings, while the rest possess low virulence.

The organisms readily change their antigenic properties. Type II pneumococcus, grown on medium containing substances of type III, acquires the properties of type III pneumococcus. This transformation from one type to another is caused by DNA.

Resistance. Pneumococci live in dried sputum for 2 months and on artificial media for 4-7 days. At 50-60°C they are destroyed within 10 minutes and in 3 per cent phenol within 1-5 minutes. In vitro they are sensitive to optochin in concentrations of up to 1 part in 2,000,000. The organisms are also sensitive to bile and bile salts.

Pathogenicity for animals. Among farm animals, calfs, piglets, lambs, and in vivariums, guinea pigs, may suffer from pneumonia. Among experimental animals white mice and rabbits are most susceptible to pneumococci, the resulting infection causing septicaemia, enlargement of the spleen, and the appearance of fibrinous exudate at the site of inoculation. Pneumonia may be produced experimentally in monkeys by intratracheal infection.

Pathogenesis and diseases in man. The exogenic route of infection is characteristic of type I, II, and III pneumococci, and the endogenic route is characteristic of all the other types. The disease usually develops as a result of reduced body resistance (chilling, bronchitis, influenza, overstrain, and other unfavourable factors).

Pneumococci of the first three types are responsible for lobar pneumonia in humans. The disease is characterized by an acute and cyclic course: the stage of blood congestion and its exudation into the alveolar lumen, stage of red hepatization during which the exudate in the alveoli coagulates, stage of grey hepatization marked by erythrocyte disintegration and leucocyte infiltration, and stage of exudate resolution-resorption.

Pneumococci may also cause other pathological conditions: septicaemia, meningitis, lesions of the joints, ulcerative endocarditis, otitis, peritonitis, rhinitis, highmoritis, serpiginous ulcer of the cornea, tonsillitis and acute catarrh of the upper respiratory tract.

It should be borne in mind that pneumonia may arise as a result of infection with staphylococci, streptococci, influenza bacteria, mycoplasmas (*Mycoplasma pneumoniae*), Chlamydozoaceae (*Miyagawanella pneumoniae*), influenza virus, and other microorganisms.

Immunity. Pneumonia and other pneumococcal diseases leave no stable or prolonged immunity. The organisms often sensitize the patient's body to reinfections. The relatively low grade of postinfectional immunity is accounted for by low immunogenic activity of the pneumococci and also by the presence of a large variety of types which produce no cross immunity.

Immunity to pneumococcal infections depends on the full value of the general physiological defense mechanisms of the body. During the disease phagocytosis is intensified. This process is, in its turn, dependent on the presence of antibodies, in particular opsonins and antibodies against capsular substances responsible for virulence. Chilling, overstrain, and malnutrition enhance susceptibility to the nonpathogenic pneumococci. The latter are normally present in the body but become pathogenic when the natural defense mechanisms of the human body are weakened.

Laboratory diagnosis. Test material includes sputum, exudates, pus, blood, spinal fluid, and organs obtained at autopsy. The diagnosis is carried out in several successive stages:

(1) microscopy of sputum for detecting capsular Gram-positive pneumococci;

(2) isolation of a pure culture by seeding sputum or pus on blood, serum, or ascitic agar and blood in serum or sugar broth; identification of the culture according to its biochemical, serological, and biological properties; simultaneously the tested material is injected subcutaneously or intraperitoneally into white mice (for pure culture isolation);

(3) typing of pneumococci by the agglutination test; special

methods for quick results are applied: (a) Neufeld's phenomenon (swelling of capsules); (b) Sebin's method (inoculation of white mice with the material under test and subsequent microagglutination of the abdominal exudate with type sera);

(4) determination of sensitivity of the isolated culture to antibiotics.

Treatment. Pneumonia patients are prescribed antibiotics (penicillin, chlortetracycline, oxytetracycline, albomycin, chloromycetin) and sulphonamides (streptocid, norsulphazol, methylsulphazine, sulphadimezin) which have replaced completely the antipneumococcal sera. The use of these drugs has provided a significant decrease in lethality and a less severe course of the disease.

New preparations of penicillin are used in the presence of penicillin-resistant pneumococci.

Prophylaxis. Protection from severe chilling, raising of body resistance by physical culture, training and hardening, observance of a normal sanitary procedures in everyday life and during work are of great importance in prevention of pneumococcal diseases. There is no need to elaborate specific measures for the prevention of pneumococcal disease, as there are many serologic types which do not produce cross immunity and pneumococci cause no epidemic outbreaks.

MENINGOCOCCI

The meningococcus (*Neisseria meningitidis*) was isolated from the cerebrospinal fluid of patients with meningitis and studied in detail in 1887 by A. Weichselbaum. At present the organism is classified in the genus *Neisseria*, family *Neisseriaceae*.

Morphology. The meningococcus is a coccus 0.6-1 μ in diameter, resembling a coffee bean, and is found in pairs. The organism is Gram-negative. As distinct from pneumococci, meningococci are joined longitudinally by their concave edges while their external sides are convex. Spores, capsules and flagella are not formed. In pure cultures meningococci occur as tetrads (in fours) and in pus they are usually found within (Fig. 81*a*) and less frequently outside the leucocytes.

In culture smears, small or very large cocci are seen singly, in pairs, or in fours. Meningococci may vary not only in shape but also in their Gram reaction. Gram-positive diplococci appear among the Gram-negative cells in smears.

Cultivation. The meningococcus is an aerobe and does not grow on common media. It grows readily at pH 7.2-7.4 on media to which serum or ascitic fluid has been added. Optimum temperature for growth is 36-37°C and there is no growth at 22°C. On solid media the organisms form fine transparent colonies measuring 2-3 mm in diameter (Fig. 81*c*). In serum broth they produce turbidity and a

precipitate at the bottom of the test tube, and after 3-4 days, a pellicle is formed on the surface of the medium.

Meningococci can be adapted to simple media by repeated subculture on media with a gradual change from the optimum protein concentration to media containing a minimal concentration of proteins.

Fermentative properties. Meningococci do not liquefy gelatin, cause no change in milk, and ferment glucose and maltose, with acid formation.

Toxin production. Meningococci produce toxic substances which possess properties of exo- and endotoxins. Disintegration of bacterial cells leads to the release of a highly toxic endotoxin. Meningococci readily undergo autolysis which is accompanied by accumulation of toxins in the medium. The meningococcal toxin is obtained by treating the bacterial cells with distilled water, or 10 N solution of soda, by heat autolysis, by exposure to ultraviolet rays.

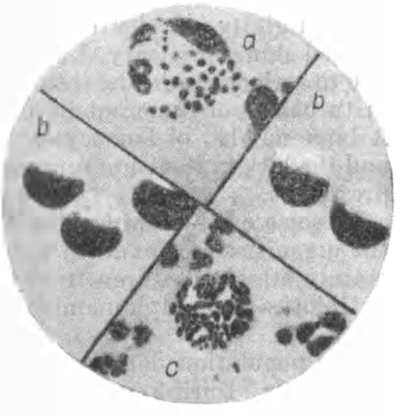


Fig. 81. *a*—meningococci; *b*—gonococci; *c*—colonies of meningococci on serum agar

Antigenic structure and classification. Meningococci were found to contain three fractions: carbohydrate (C) which is common to all meningococci, protein (P) which is found in gonococci and type III pneumococci, and a third fraction with which the specificity of meningococci is associated. According to the International Classification, four groups of meningococci are distinguished, groups A, B, C, and D. Recently the number of types has increased to seven, but only the first two are dominant.

The organisms are characterized by intraspecific variability. A change of types takes place at certain times.

Resistance. The meningococcus is a microbe of low stability, and is destroyed by drying in a few hours. By heating to a temperature of 60°C it is killed in 10 minutes, and to 80°C, in 2 minutes. When treated with 1 per cent phenol, the culture dies in 1 minute. The organism is very sensitive to low temperatures. Bearing this in mind, test material should be transported under conditions which protect the meningococcus against cooling.

Pathogenicity for animals. Animals are not susceptible to the meningococcus in natural conditions. The disease can be produced experimentally in monkeys and rabbits by subdural injections of

meningococci. Intrapleural and intraperitoneal infection of guinea pigs and mice results in lethal intoxication. Septicaemia develops in experimental animals only when large doses are injected.

Pathogenesis and diseases in man. People suffering from meningococcal infection and carriers are sources of diseases. The infection is transmitted by the air droplet route. The causative agent is localized primarily in the nasopharynx. From here it invades the lymph vessels and blood and causes the development of bacteraemia. Then as a result of metastasis the meningococci pass into the meninges and produce acute pyogenic inflammation in the membranes of the brain and spinal cord.

The disease usually arises suddenly with high temperature, vomiting, rigidity of the occipital muscles, severe headache, and increased skin sensitivity. Later paresis of the cranial nerves develops due to an increase in the intracranial pressure. Dilation of the pupils, disturbances of accommodation, as well as other symptoms appear. A large number of leucocytes are present in the cerebrospinal fluid, and the latter after puncture escapes with a spurt because of the high pressure.

In some cases meningococcal sepsis develops. In such conditions the organisms are found in the blood, joints, and lungs. The disease mainly attacks children from 1 to 5 years of age. Before the use of antibiotics and sulphonamides the death rate was very high (30-60 per cent).

The population density plays an important part in the spread of meningitis. During epidemic outbreaks there is a large number of carriers for every individual affected by the disease. In nonepidemic periods the carrier rate increases in the spring and autumn. Body resistance and the amount and virulence of the causative agent are significant. Depending on these factors, the spread of infection is either sporadic or epidemic.

Meningitis can also be caused by other pathogenic microbes (streptococci, pneumococci, staphylococci, bacteria of influenza, mycobacteria of tuberculosis, and certain viruses). These organisms, however, cause sporadic outbreaks of the disease, while meningococci may cause epidemic meningitis.

Immunity. There is a well-developed natural immunity in humans. Acquired immunity is obtained not only as a result of the disease but also as the result of natural immunity developed during the meningococcal carrier state. In the course of the disease agglutinins, precipitins, opsonins, and complement-fixing antibodies are produced. Recurring infections are rare.

Laboratory diagnosis. Specimens of cerebrospinal fluid, nasopharyngeal discharge, blood, and organs obtained at autopsy are used for examination.

The following methods of investigation are employed: (1) microscopic examination of cerebrospinal fluid precipitate; (2) inoculation

of this precipitate, blood or nasopharyngeal discharge into ascitic broth, blood agar, or ascitic agar; identification of the isolated cultures by their fermentative and serologic properties; differentiation of meningococci from the catarrhal micrococcus (*Neisseria catarrhalis*) and saprophytes normally present in the throat. The meningococcus ferments glucose and maltose, whereas *Neisseria catarrhalis* does not ferment carbohydrates, and *Neisseria sicca* ferments glucose, levulose, and maltose; (3) performance of the precipitin reaction with the cerebrospinal fluid.

Treatment. Antibiotics (penicillin, chlortetracycline, oxytetracycline, etc.) and sulphonamides (streptocid, methylsulphazine) are prescribed.

Prophylaxis is ensured by general sanitary procedures and epidemic-control measures (early diagnosis, transference of patients to hospital), appropriate sanatory measures in relation to carriers, quarantine in children's institutions). Observance of hygiene in factories, institutions, public premises, and lodgings, and prevention of crowded conditions are also obligatory. Vaccination is not employed. The wide use of sulphonamides and antibiotics in postwar years has reduced the incidence of epidemic meningitis to sporadic cases and the mortality rate by 33.4-50 per cent. Only in African countries as a result of poor living conditions, the morbidity rate is still high and is characterized by epidemic rises up to 46-54 cases per 10,000 of the population. The mortality rate in these countries reaches approximately 20 per cent.

GONOCOCCI

The causative agent of gonorrhoea and blennorrhoea (*Neisseria gonorrhoeae*) was discovered in 1879 by A. Neisser in suppurative discharges. In 1885 E. Bumm isolated a pure culture of the organism and studied it in detail. Gonococci belong to the genus *Neisseria*, family *Neisseriaceae*.

Morphology. Gonococci are morphologically similar to meningococci. The organism is a paired, bean-shaped coccus, measuring 0.6-1 μ in diameter. It is Gram-negative and occurs inside and outside cells. Neither spores nor flagella are formed. Under the electron microscope a cell wall, 0.35-0.4 μ in thickness, surrounding the gonococci is visible.

Pleomorphism of the gonococci is a characteristic property. They readily change their form under the influence of medicines, losing their typical shape, and growing larger, sometimes turning Gram-positive, and are found outside the cells.

In chronic forms of the disease autolysis of the gonococci takes place with formation of variant types (Asch types). Usually gonococcal cells, varying in size and shape, are formed. The tendency to-

wards morphological variability among the gonococci should be taken into account in laboratory diagnosis.

Cultivation. The gonococcus is an aerobe, which does not grow on ordinary media, but can be cultivated readily on media containing human proteins (blood, serum, ascitic fluid) when the pH of the media is in the range of 7.2-7.6. The optimal temperature for growth is 37°C, and the organism does not grow at 25 and 42°C. It also requires an adequate degree of humidity. Ascitic agar, ascitic broth, and egg-yolk medium are the most suitable media. On solid media gonococci produce transparent, circular colonies, 1-3 mm in diameter. Cultures of gonococci form a pellicle in ascitic broth, which in a few days settles at the bottom of the test tube.

Fermentative properties. The gonococcus possesses low biochemical activity and no proteolytic activity. It ferments only glucose, with acid formation.

Toxin production. The gonococci produce no soluble toxin (exotoxin). An endotoxin is released as a result of disintegration of the bacterial cells. This endotoxin is also toxic for experimental animals.

Antigenic structure and classification. The antigenic structure of gonococci is associated with the protein and polysaccharide fractions. The organisms contain four group-specific (A, B_{1-4}, C, and D) and three type-specific (E, F, and G) antigens. Antigens B_{1-4} and D are common to gonococci and meningococci.

Resistance. Gonococci are very sensitive to cooling. They do not survive drying, although they may live as long as 24 hours in a thick layer of pus or on moist objects. They are killed in 5 minutes at a temperature of 56°C, and in several minutes after treatment with a 1 : 1,000 silver nitrate solution or 1 per cent phenol.

Pathogenicity for animals. Gonococcus is not pathogenic for animals. An intraperitoneal injection of the culture into white mice results in fatal intoxication but does not produce typical gonorrhoea.

Pathogenesis and diseases in man. Patients with gonorrhoea are sources of the infection. The disease is transmitted via the genital organs and by articles of domestic use (diapers, sponges, towels, etc.). The causative agent enters the body via the urethral mucous membranes and, in women, via the urethra and cervix uteri. Gonorrhoea is accompanied by acute pyogenic inflammation of the urethra, cervix uteri. and glands in the lower genital tract. Often, however, the upper genito-urinary organs are also involved. Inflammations of the uterus, uterine tubes, and ovaries occur in women, vulvovaginitis occurs in girls, and inflammation of the seminal vesicles and prostata in men. The disease may assume a chronic course. From the cervix uteri the gonococci can penetrate into the rectum. Inefficient treatment leads to affections of the joints and endocardium, and to septicaemia. Gonococcus is responsible for gonorrhoeal conjunctivitis and blennorrhoea in adults and newborn infants.

Immunity. The disease does not produce insusceptibility and there is no congenital immunity. Antibodies (agglutinins, precipitins, opsonins, and complement-fixing bodies) are present in patients' sera, but they do not protect the body from reinfection and recurrence of symptoms. Phagocytosis in gonorrhoea is incomplete. The phagocytic and humoral immunity produced in gonorrhoea is incapable of providing complete protection, so, in view of this fact, treatment includes measures which increase body reactivity. This is achieved by raising the patient's temperature artificially.

Laboratory diagnosis. Specimens for microscopic examination are obtained from the discharge of the urethra, vagina, vulva, cervix uteri, prostata, rectal mucous membrane, and conjunctiva. The sperm and urine precipitates and filaments are also studied microscopically. Smears are stained by Gram's method and with methylene blue by Loeffler's method (see Fig. 117,7). Microscopy is quite frequently an unreliable diagnostic method since other Gram-negative bacteria, identical to the gonococci, may be present in the material under test. Most specific are the immuno-fluorescence methods (both direct and indirect). In the direct method the organisms under test are exposed to the action of fluorescent antibodies specific to gonococci. In the indirect method, the known organisms (gonococci) are treated with patient's serum. The combination of the antibody with the antigen becomes visible when fluorescent antiserum is added (see "Practical Manual").

If diagnosis cannot be made by microscopic examination, isolation of the culture is carried out. For this purpose the test material (pus, conjunctival discharge, urine precipitate, etc.) is inoculated onto media. The Bordeux-Gengou complement-fixation reaction and the allergic test are employed in chronic and complicated cases of gonorrhoea.

Treatment. Patients with gonorrhoea are prescribed antibiotics (penicillin, chlortetracycline, and polymyxin) and sulphonamides (streptocid, norsulphazol, and sulphacil). Injections of polyvalent vaccine and autovaccine as well as pyrotherapy (introduction of heterologous proteins) are applied in complicated cases.

Improper treatment renders the gonococci drug-resistant, and this may lead to the development of complications and to a chronic course of the disease.

Prophylaxis includes systematic precautions for establishing normal conditions of everyday and family life, health education and improvement of the general cultural and hygienic standards of the population. In the control of gonorrhoea great importance is assigned to early exposure of sources of infection and contacts and to successful treatment of patients.

The prevention of blennorrhoea is effected by introducing one or two drops of a 2 per cent silver nitrate solution into the conjunctival sac of all newborn infants. In certain cases (in prematurely born

infants) silver nitrate gives no positive result. Good results are obtained by introducing two drops of a 3 per cent penicillin solution in oil into the conjunctival sac. The gonococci are killed in 15-30 minutes.

In spite of the use of effective antibiotics the incidence of gonorrhoea tends to be on the increase in all countries (Africa, America, South-Eastern Asia, Europe, etc.). The number of complications has also increased: gonococcal ophthalmia of newborn infants (blennorrhoea), vulvovaginitis in children, and inflammation of the pelvic organs (salpingitis) and sterility in women. The rise in the incidence of gonorrhoea is caused by social habits (prostitution, homosexualism, etc.), inefficient registration of individuals harbouring the disease (not exceeding 1 per cent), deficient treatment, and the appearance of gonococci resistant to the drugs used.

The WHO expert committee has recommended listing the gonococcal infection among infectious diseases with compulsory registration and making a profound study of the cause of the epidemic character of gonococcal diseases in certain African countries. Stricter blennorrhoea control measures, and elaboration of uniform criteria of clinical and laboratory diagnosis and treatment of gonococcal infection and more efficient methods for determining the sensitivity of circulating gonococci to various drugs are also recommended by the committee.

CONDITIONALLY PYOGENIC PATHOGENIC MICROBES

Suppuration may be due not only to pathogenic cocci but also to conditionally pathogenic microbes—tetracoccus, the blue pus bacterium, proteus, etc.

1. **Tetracoccus** (*Gaffkya tetragena*) (G. Gaffky, 1883) occurs in fours and has a diameter of 0.6-0.8μ (Fig. 82*a*). Quite frequently the cells are surrounded by a capsule. The organism is Gram-negative, grows readily on all media at room temperature, and does not liquefy gelatin. It is pathogenic for white mice.

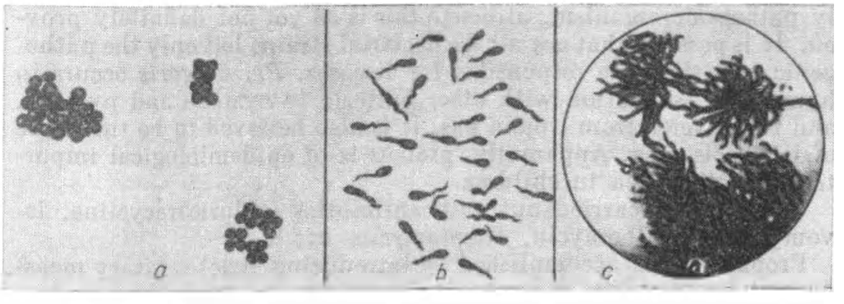


Fig. 82. *a—Gaffkya tetragena; b—Pseudomonas aeruginosa; c—Proteus vulgaris*

The tetracoccus is normally saprophytic, but it occurs frequently in the mouth where it produces suppurative processes, alveolar abscesses, affects the respiratory tract, and causes lung abscesses. Its disease-producing effect is associated with the activity of other microbes. Tetracoccus may sometimes be the aetiological factor of pneumonia and septicaemia. Treatment is carried out with penicillin and tetracycline preparations.

2. **Pseudomonas pyocyanea** (*Pseudomonas aeruginosa*) was discovered in 1895 by V. Migula. It is a small, motile, lophotrichous rod,

1.5-3μ in length (Fig. 82*b*). The organism is Gram-negative, nonsporing, and aerobic. It produces blue (pyocyanin), yellow (hemicyanin), greenish-yellow (fluorescin), and sometimes red (pyorubin) and black (melanin) pigments. In cultures it yields a mucous film and a green pigment which colours the nutrient media, pus, and faeces green. The organism liquefies gelatin. It is pathogenic for rabbits and gives rise to suppurative conditions, haemorrhagic oedema, and septicaemia. It occurs frequently in association with other organisms (staphylococci), particularly in suppurative wounds. Suppurative conditions, resulting from *Ps. aeruginosa* infection, are accompanied by fever, skin eruptions, diarrhoea, cystitis, and pyelitis. Diseases are treated with polymyxin and neomycin.

3. **Proteus** (*Proteus vulgaris*) was discovered in 1885 by G. Hauser. It is a polymorphous, motile (the O-form is nonmotile), peritrichous Gram-negative rod (Fig. 82*c*). The organism forms neither spores nor capsules, grows at temperatures between 25 and 37°C, liquefies gelatin and coagulates serum. Produces hydrogen sulphide, ammonia, and indole. Reduces nitrates to nitrites and ferments levulose, glucose, galactose, saccharose, and maltose, with acid and gas formation. Proteus is a facultative aerobe and grows readily on common media. The H-form is characterized by creeping growth. Proteus plays an important role in putrefactive processes owing to its ability to produce proteolytic enzymes.

Numerous investigators consider the bacterium to be a conditionally pathogenic organism, although this is as yet not definitely proven. It is possible that not all the bacterial strains but only the pathogenic variations are responsible for diseases. *Pr. vulgaris* occurs in humans in association with other bacteria in cystitis and pyelitis, and is recovered from wound pus. It is also believed to be the cause of food poisoning. Apparently, proteus is of epidemiological importance in diarrhoea in children.

Treatment is carried out with antibiotics (chlortetracycline, levomycetin, synthomycin, streptomycin, etc.).

Prophylaxis is accomplished by introducing strict sanitary measures at food factories and enforcing sanitary regulations during processing, storage, and transportation of foodstuffs.

The association of pyogenic cocci with a number of other pathogenic organisms (causative agents of gaseous anaerobic infection, certain viruses) is of great importance in human pathology. For example, joint action of pathogenic clostridia, staphylococci, and streptococci results in gaseous anaerobic infection. Aerobic cocci reduce the oxidation-reduction potential of the tissues and thus create more favourable conditions for the development of anaerobic infection. Alpha-haemolytic streptococci in association with adenoviruses produce chronic tonsillitis. Associations of conditionally pathogenic streptococci, pneumococci, haemoglobinophylic bacteria of influenza, adenoviruses, and other bacteria also play a definite role in the

pathogenesis of tonsillitis and catarrh of the upper respiratory tract. It may be presumed that viruses, along with the haemolytic streptococcus, play a role in the aetiology of scarlet fever and rheumatic fever.

The pyogenic cocci are quite frequently responsible for secondary infections in influenza, smallpox, measles, scarlet fever, adenovirus infections, and many other diseases.

Under conditions of low immunobiological resistance, streptococci and staphylococci are constantly present in the pharynx and tonsils of children and adults, and the concomitant disintegration of the affected tissues produces autosensitization of the body, which leads to increased sensitivity.

CAUSATIVE AGENTS OF PLAGUE, TULARAEMIA, AND BRUCELLOSIS

PASTEURELLA PESTIS

The causative agent of plague, *Pasteurella pestis*, was discovered by the French microbiologist A. Yersin in Hong Kong in 1894. The microbe was named *Pasteurella* in honour of L. Pasteur who in 1879 discovered the organism responsible for chicken cholera. The aetiological agents of human plague, chicken cholera, haemorrhagic septicaemia of cattle, and pasteurellosis of buffaloes, calfs, sheep, goats, pigs, and rodents all belong to the genus *Pasteurella*. Pasteurella organisms are widespread in nature, and can be found not only in the body of a sick animal but also in the body of a healthy carrier.

Morphology. The plague bacillus, as seen in tissue smears, is ovoid-shaped, 1-2μ in length and 0.3-0.7μ in breadth (Fig. 83). It is non-motile, forms no spores, and on solid media cultures is elongated in form.

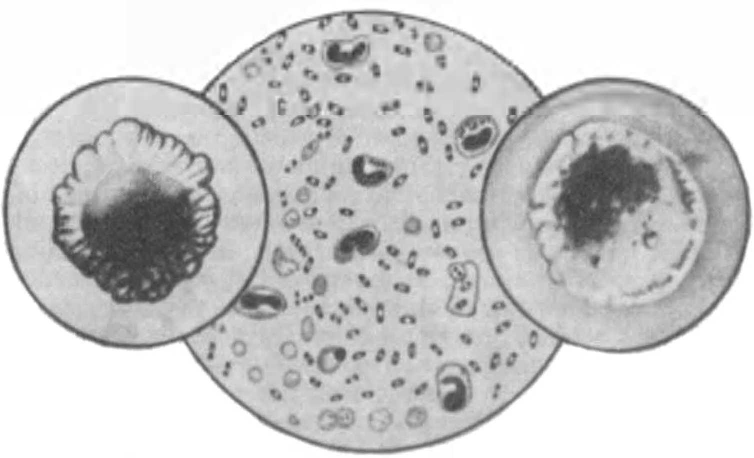


Fig. 83. *Pasteurella pestis* recovered from a bubo and colonies of *P. pestis* on meat-peptone agar

In preparations from tissues and cultures *P. pestis* is found to have a delicate capsule. The organism stains with ordinary aniline dyes and gives a bipolar appearance, its ends staining more intensively. It is Gram-negative.

P. pestis is characterized by marked individual variability (pleomorphism). In smears from organs and in young cultures it has an ovoid shape, while in cultures on solid media it is elongated and sometimes thread-like. If common salt is added to agar the bacillus shows various forms: ovoid, club-shaped, thread-like, and granular. These forms are usually known as involution forms. The occurrence of filterable types of *P. pestis* has also been demonstrated.

Cultivation. *P. pestis* is an aerobe but can also grow under anaerobic conditions. It is cultivated on ordinary media with pH 6.9-7.0. The optimal temperature for cultivation is 25-30°C. The pathogen can also grow at temperatures ranging from 0° to 45°C and at pH from 5.8 to 8.0.

On agar slants the culture forms a viscid translucent mucilaginous mass. On agar plates it forms colonies with turbid white centres, and scalloped borders (see Fig. 83) resembling lace or crumpled lace handkerchiefs.

In meat broth the cultures form a pellicle on the surface with thread-like growth resembling stalactites and a flocculent precipitate.

Sodium sulphite, fresh haemolytic blood, sarcinic extract, and live sarcina ("feeders") are used as growth stimulators. They are of special value when the seeded material contains a small number of organisms.

P. pestis possesses intraspecific variability. It changes quite easily from the virulent R-forms to avirulent S-forms through the O-forms.

Resistant S-forms develop in the presence of bacteriophage. They closely resemble the *Pasteurella pseudotuberculosis* of rodents. Vaccine strains of the plague bacillus are of great practical value and are used for preparing live vaccines.

Fermentative properties. *P. pestis* does not liquefy gelatin, nor does it produce indole. It reduces nitrates to nitrites, ferments glucose, levulose, maltose, galactose, xylose, mannitol and, occasionally, arabinose, with acid formation. Some strains ferment glycerin, while others do not. The differential diagnosis of *P. pestis* and *P. pseudotuberculosis* is very difficult. Unlike *P. pseudotuberculosis* of rodents, *P. pestis* does not break down adonitol and rarely ferments rhamnose and lactose (Table 16).

Toxin production. *Pasteurella pestis* is extremely virulent for human beings. It produces a toxin of high potency which has the properties of both exo- and endotoxins and is capable of causing erythrocyte haemolysis and fibrin resolution. The continental strains (see p. 180) produce urease, a toxic substance. The toxin has been obtained in its pure form as an active preparation containing nitrogen, phosphorus, sulphur, and carbohydrates. It is highly toxic;

Table 16

Differential Characteristics of *P. pestis* and *P. pseudotuberculosis*

Pasteurella pestis	*Pasteurella pseudotuberculosis*
Lysed by all types of plague bacteriophage	Lysed only by certain types of plague bacteriophage
Possesses fibrinolytic properties	Does not possess fibrinolytic properties
Fresh strains usually do not ferment rhamnose	Fresh strains normally ferment rhamnose
Does not ferment adonitol	Ferments adonitol, with acid formation
The R-form is virulent, the S-form is avirulent	The S-form is more virulent than the R-form
Highly pathogenic for guinea pigs, less pathogenic for rabbits	Less pathogenic for guinea pigs, highly pathogenic for rabbits
Agglutinated by antiplague serum to the titre, is not agglutinated by pseudotuberculosis S-serum	The R-form is agglutinated by antiplague serum, usually to the titre, the S-form shows little or no agglutination by antiplague serum
Shows no growth on non-nutrient (containing no peptone) 3 per cent agar	Shows a faint growth on non-nutrient 3 per cent agar

there are 86,000 lethal mouse doses per 1 mg of nitrogen in the preparation.

Antigen structure. *Pasteurella pestis* possesses 10 antigens, among which are a specific capsular thermolabile (polysaccharide) A-antigen and a nonspecific thermostable (protein) B-antigen. The capsular substance is associated with the immunogenic activity of vaccines. Owing to this phenomenon the method of producing antiplague serums by hyperimmunization of horses with the polysaccharide specific complex antigen, obtained from the capsular substance of *P. pestis*, was elaborated. Besides the capsular and somatic antigens a surface somatic antigen is present in the body of the plague organism. By the agar precipitation method *P. pestis* was found to possess antigens common to bacteria of the enteric fever and dysentery groups.

Classification. According to the present classification, the aetiological agent of plague belongs to the order *Eubacteriales*, family *Brucellaceae*, genus *Pasteurella*.

There are two varieties of *Pasteurella pestis*. One of the varieties ferments glycerin, produces urease, possesses a specific antigen, and is isolated in continental plague foci. Hibernating rodents (marmots, susliks) are reservoirs of this variety. The other variety (ocean) does not ferment glycerin, does not possess urease activity, contains no antigens characteristic of the first variety, and is isolated in rat-spread foci. The main sources of this variety of *P. pestis* are

rodents of the mouse family. Strains with intermediate properties also occur.

Resistance. The plague bacillus can withstand low temperatures. At 0° C it lives for 6 months. It survives on clothes for 5-6 months; in sterile soil and in milk, for 90 days; in grain and on cadaver, for 40 days; in water, for 30 days; in bubo pus, for 20-30 days; in sputum, for 10 days; in vegetables and fruits, for 6-11 days; and in bread, for 4 days.

P. pestis is very sensitive to drying and high temperatures. Boiling kills the organism within 1 minute, and when heated to 60°C it is destroyed in 1 hour. In a 5 per cent phenol solution it is killed in 5-10 minutes and in a 5 per cent solution of lysol, in 2-10 minutes.

Pathogenicity for animals. Rodents, among them black rats, grey rats, mice, susliks, midday gerbils, tumarisks, and marmots (tarbagans) are susceptible to plague. More than 300 rodent species may spontaneously contract the disease. In addition, 19 rodent species are susceptible to laboratory infection with plague. Camels died in the Astrakhan steppes in 1911, and humans who ate camel meat contracted plague. Pigs, sheep, goats, donkeys, mules, dogs, cats, monkeys, and certain carnivores are susceptible to the disease in natural environments. However, little epidemiological importance is attached to them.

Guinea pigs, white mice, white rats, and rabbits are the experimental animals which easily acquire the infection. Animals experimentally inoculated with plague display sepsis, necrosis at the site of injection, enlargement of lymph nodes and spleen, haemorrhages in the skin and mucous membranes (haemorrhagic septicaemia).

Pathogenesis and disease in man. In the past plague was a menace, causing the devastation of entire nations. In the VI century about hundred million people died within a period of 50 years. The population of the Eastern Roman Empire suffered from the disease particularly severely. A pandemic of plague broke out in the XIV century and was known at that time as the "great" or "black death". As a result of this outbreak 25 million people died in Europe (25 per cent of the population) and 35 million in Asia.

From 1894 to 1938 plague caused the death of more than 13 million people in various countries throughout the world.

Plague morbidity did not cease in the following years. The disease also occurs today, particularly in the endemic regions of Asia and Africa. According to data furnished by WHO (World Health Organization) 2,541,461 cases of plague were recorded in all the countries of the world between 1921 and 1950, 28,323 cases, between 1951 and 1961, and 5,382 cases, between 1961 and 1964.

Plague is a zoonotic, transmissible, particularly hazardous disease with natural nidality. Its causative agents, vectors, and animals (reservoirs of the pathogen) can survive in nature for an unlimited period of time. Contamination with plague occurs usually through

rodent fleas, rodent urine, and flea faeces. The black rat flea (*Xenopsylla cheopis*) and grey rat flea (*Ceratophyllus fasciatus*) cannot swallow blood when infected with plague since the entrance to their stomach (proventriculus) is blocked by plague bacteria. When such a flea feeds again, part of the ingested blood, together with the plague microbes, is regurgitated. The human flea can also be a vector of the causative agent of plague.

Among animals of the steppe, marmots (tarbagans) and susliks are reservoirs of *P. pestis*. They hibernate from late summer or early autumn. During hibernation the development of the infection in the contaminated rodents is considerably retarded, the body temperature falls sharply, and the body resistance is reduced. During this period rodents do not acquire immunity. For this reason, being carriers of plague microbes, they may contract the disease themselves and infect their young in spring.

The current epidemiology of plague was scientifically founded by D. Zabolotny, G. Minkh, I. Deminsky, N. Klodnitsky, and many others. From vast amounts of data they ascertained the role of wild rodents (susliks, tarbagans) as harbourers and sources of the plague bacilli in nature. As a result of the work of these scientists, an effective system of antiplague measures was organized. The practice of these measures contributed to the elimination of plague epidemics in the USSR.

P. pestis enters the human body through abrasions in the skin (sometimes through the mucous membranes). In the pneumonic form of the disease the plague bacilli are spread in the air with sputum expelled by the patient when he coughs or talks. Quite often no inflammation is seen at the site of microbe entry. Depending on the location of the pathogen, reactivity of the infected body, virulence of the microbe, and extent of cellular and humoral activity, human plague may be of the following forms; cutaneous, bubonic, cutaneous-bubonic, primary septicaemic, secondary septicaemic (primary pneumonic), secondary pneumonic, and intestinal.

As a rule, plague develops quickly without a prodromal stage. It is characterized by a violent chill, severe headache, and dizziness. The face is pale and bluish, with an expression of suffering (horror-stricken), and is known as *facies pestica*. Each form of plague shows characteristic clinical symptoms. Before the use of streptomycin the mortality rate was very high, i. e., from 40 to 100 per cent. Post-mortem examination reveals centres of inflammation in the lymph nodes, haemorrhages, and haemorrhagic periadenitis. A large number of *P. pestis* organisms are present in the lymph nodes and cellular tissue. Phagocytosis is inhibited. There is marked disintegration and necrosis of the buboes. Small haemorrhages form in the skin. The liver is enlarged, showing haemorrhages and necrosis. The spleen is enlarged and dark-red in colour.

The pneumonic foci fuse and assume the form of lobar pneumonia.

The lungs are distended, violet-red or grey-red in colour, hyperaemic, oedematous, and contain a large number of *P. pestis* organisms.

Immunity. After recovery from the disease a stable immunity of long duration is acquired. Realizing this, in ancient times people living in countries invaded by plague made use of convalescents for nursing plague patients and burying corpses.

Postinfection and postvaccinal immunities are predominantly of the phagocytic type. Of particular importance are opsonins which facilitate the phagocytic activity of cells of the lymphoid-microphage system.

Laboratory diagnosis. Examination is carried out in special laboratories and in antiplague protective clothing. A strict work regimen must be observed. Depending on the clinical form of the disease and the location of the causative agent, test specimens are collected from bubo content (in bubonic plague), ulcer secretions (in cutaneous plague), mucus from the pharynx and sputum (in pneumonic plague), and blood (in septicaemic plague). Test matter is also recovered from necropsy material (organs, blood, lungs, contents of lymph nodes), rodent cadavers, fleas, foodstuffs, water, air, etc. Examination is performed in the following stages.

1. Microscopy of smears, fixed in Nikiforov's mixture and stained by the Gram method or with methylene blue by Loeffler's method.

2. Inoculation of the test material into nutrient media, isolation of a pure culture and its identification. To inhibit the growth of the accompanying microflora, 1 ml of a 2.5 per cent sodium sulphite solution and 1 ml of a concentrated alcohol solution of gentian violet, diluted in distilled water in a ratio of 1:100, are added to 100 ml of meat-peptone agar. Prior to inoculation 0.1 ml of antiphage serum is added to the culture to render the plague bacteriophage harmless.

3. Biological tests of the isolated pure culture and of material from which isolation of the organism is difficult are conducted on guinea pigs. In the latter case a thick emulsion prepared from the test material is rubbed into a shaven area of skin on the abdomen. If plague bacilli are present the animals die on the fifth-seventh day. To hasten diagnosis the infected guinea pigs are killed on the second-third day and the plague bacillus is isolated from their organs.

P. pestis is identified by determining the morphological, cultural, fermentative, phagocytolytic, and agglutinative properties of the isolated culture. The growth is differentiated from the causative agent of rodent pseudotuberculosis (see Table 16). The biological test is decisive in plague diagnosis.

Decomposed rodent cadavers are examined by the thermoprecipitin test.

The importance of prompt diagnosis of plague has led to the elaboration of accelerated diagnostic methods in recent times.

Treatment. At present streptomycin is used for treatment of plague, the drug being very effective and curing even pneumonic plague

in a high per cent of cases. Good results have been obtained from a combination of streptomycin with chloromycetin or tetracycline with antiplague serum. Antiplague serum or antiplague gamma-globulin and a specific bacteriophage are also used for treatment of plague patients. Penicillin, chlortetracycline or albomycin, and sulphonamides are recommended in cases with complications.

Prophylaxis. General prophylaxis comprises the following measures:

(1) early diagnosis of plague, particularly the first cases;

(2) immediate isolation and hospitalization of patients and enforcement of quarantine; individuals who have been in contact with patients are placed under quarantine for 6 days and prescribed prophylactic streptomycin treatment;

(3) observation (i. e., isolation of individuals or groups of people suspected of having been in contact with infected material, daily inspection from house to house, thermometry twice a day, and observation during the possible incubation period);

(4) thorough disinfection and extermination of rats in disease foci;

(5) individual protection of medical personnel and prophylactic treatment with streptomycin and vaccination;

(6) prophylactic measures and systematic observation carried out by plague control laboratories, stations, and institutes in endemic areas;

(7) observance of international plague control conventions (extermination of rats and disinfestation of ships, aircraft, trains, and harbours and, if necessary, compulsory quarantine for passengers);

(8) security measures from plague invasion at frontiers.

Specific prophylaxis is accomplished with live EV vaccine.

In the USSR the antiplague vaccine is prepared from strain-1 and strain-17. It is produced in dry matter and introduced subcutaneously, intracutaneously, or by rubbing into the skin, in one or two doses. Immunity lasts for about a year. Depending on epidemiological conditions, revaccination is carried out within 6 or 12 months.

CAUSATIVE AGENT OF TULARAEMIA

The specific cause of tularaemia (*Francisella tularensis*) was discovered in 1912 by G. McCoy and C. Chapin in Tulare (California) and studied in detail by E. Francis.

Morphology. The tularaemia bacteria are short coccal-shaped or rod-like cocci (Fig. 84) measuring 0.2-0.7 μ. In old cultures the organisms retain the coccal form. They are nonmotile, polychromatophilic, and Gram-negative. In the animal body they are sometimes surrounded by a fine capsule.

Tularaemia bacteria are pleomorphous. They may assume a club-like structure or the form of very small cocci (0.1-0.2 μ) which pass

through filters. Average-sized cocci, very large spherical forms, or spherical forms with kidney-like protrusions are also to be found. Smears demonstrate rod-like and thread-like forms of the organism, which may reach 8μ in length. In animal organs the tularaemia bacteria are seen mainly as coccal bacteria or as rod-like forms, while in cultures coccal forms are more often observed. Cultures of low virulence (vaccine strains) are coccal-shaped, larger than those of the virulent strain, and, as a rule, do not have a capsule.

Cultivation. The tularaemia organism is an aerobe which does not grow on ordinary media, but grows well at 37° C on media rich in vitamins, e.g., yolk medium which consists of 60 per cent of yolk and 40 per cent of a 0.85 per cent sodium chloride solution with pH 6.7-7.4. The organisms are cultured in a thermostat for 2-14 days.

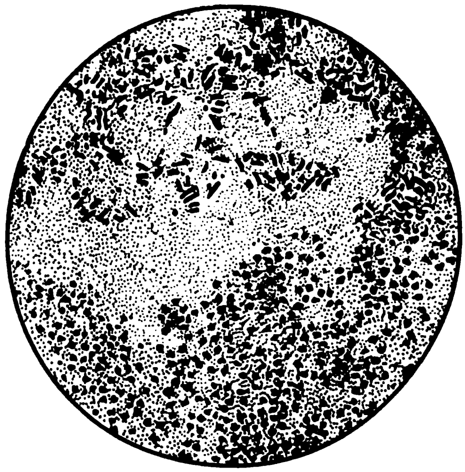


Fig. 84. The causative agent of tularaemia (coccal-shaped and rod-like forms)

Tularaemia bacteria multiply on cystine agar, containing 0.05-0.1 per cent cystine and 1 per cent glucose. The mixture is boiled for several minutes, cooled to 40-50°C, after which defibrinated blood is added. The latter should constitute 5-10 per cent of the nutrient medium. Milk-white colonies are formed. The bacteria can be cultivated on media containing brain, spleen, and liver tissues, heart extracts, brewer's yeast, and fish flour. Such media contain vitamins necessary for growth of the tularaemia organisms. Culture collections on solid media may be well preserved for 2-6 months. Tularaemia bacteria grow well in the yolk sac of a 12-day-old chick embryo.

When cultivated in the laboratory, tularaemia organisms lose the Vi-antigen with which their virulence and immunogenic properties are associated.

Fermentative properties. Tularaemia bacteria break down proteins with the elimination of hydrogen sulphide, and do not produce indole. They ferment glucose, levulose, mannose, and maltose, with acid formation. Dextrin, saccharose, and glycerin fermentation is not a stable property. Biochemical properties are unstable and liable to comparatively rapid changes. This is due not only to the proper-

ties of the bacteria themselves but also to the nutrient media and to the ability of the tularaemia organism to break down proteins. The products of this process mask the production of acids which occurs simultaneously.

Toxin production. The existence of a soluble toxin in tularaemia bacteria has not been demonstrated. The organism's virulence is associated with its Vi-antigen. The tularaemia bacterium grows poorly in liquid media, and for this reason it is difficult to isolate any toxin.

Antigenic structure. Agglutination test and precipitin reaction are highly specific. Tularaemia bacteria have been shown to possess thermostable specific haptens. The common character of the antigens of tularaemia and brucellosis agents in the agglutination reaction has been ascertained. This fact must be taken into account in serological diagnosis of these diseases.

The R-form cultures containing only the O-antigen are avirulent and possess no immunogenic properties. The S-form which contains Vi- and O-antigens is more common. The intermediate SR-forms from which live vaccines are prepared contain the O-antigen and, in a smaller number, the Vi-antigen. With prolonged growth on laboratory media all cultures transform to the R-form.

Since complete avirulence is accompanied by the loss of immunogenic properties, a certain degree of virulence for white mice is preserved (residual virulence) in tularaemia strains from which the vaccine is prepared.

Classification. Two varieties of tularaemia organisms can be distinguished: the American type which is highly pathogenic for rabbits and ferments glycerin, and the European type which is nonpathogenic for rabbits and does not ferment glycerin. The former variety is also more virulent for human beings, causing death in 5-6 per cent of tularaemia cases. The second variety was responsible for a low death rate among humans, lethality being 0.5 per cent.

Resistance. The tularaemia organism lives in glycerin for 240 days, in grain, for 130, in water and rodent cadavers, for 90, in baked bread, for 20, and in soil, for 10 days. It survives for long periods (over 3 months) at low temperatures. It is killed in several minutes by 3 per cent solutions of lysol, cresol, saponaceous cresol, formalin, and alcohol. When heated to 60°C it is destroyed in 10-15 minutes, while exposure to direct rays destroys it in 30 minutes.

Pathogenicity for animals. The organism is pathogenic for water rats, field voles, grey rats, common field mice and house mice, hares, susliks, chipmunks, hamsters, muskrats, gerbils, moles, shrews, and other animals. Among the domestic animals camels, sheep, cats, dogs, and pigs are susceptible to the disease, and among laboratory animals, guinea pigs and white mice.

Guinea pigs are inoculated intraperitoneally, subcutaneously, intracutaneously, and by rubbing the infective material into the

skin. The inoculated animals develop a fever and lassitude, and lose weight. The spleen, liver, and inguinal lymph nodes become enlarged and inflamed. Microscopic studies reveal the presence of the causative agent in the spleen, liver, bone marrow, lymph nodes and in the blood recovered from the heart.

Wide adaptivity is characteristic of the tularaemia bacterium. It has adapted itself to more than 70 species of vertebrates and 60 species of arthropods which are capable of transmitting the disease. However, water rats, field voles, mice, muskrats, and, among domestic animals, sheep, and, among the vectors, horseflies, ticks, mosquitoes, and sandflies have the most epidemiological importance.

Pathogenesis and disease in man. Tularaemia is a zoonotic disease. Human beings contract it by the air-droplet route. The pathogen may also gain entrance into the body through the integuments and mucous membranes as a result of bites by arthropods and insects (ticks, horseflies, mosquitoes, etc.).

Contingent on the route of entry, the bacteria invade the skin, mucous membranes, lymph nodes, respiratory and gastrointestinal tracts, and other organs, causing the respective clinical form of the disease (bubonic, ulcerative-bubonic, ocular, anginose-bubonic, abdominal or intestinal, pneumonic, and generalized or primary septicaemia). The lymph nodes are affected in all forms of the disease. In the generalized form all tissues and organs are involved as a result of bacteraemia.

Tularaemia may be acute, lingering, or relapsing, depending on the duration of the disease, and may be mild, severe, or mildly severe.

During tularaemia allergy develops and remains for years and sometimes for life.

In recent years the death rate is low and most patients recover owing to the wide use of antibiotics.

According to the mode of spread and route of infection, the following types of tularaemia epidemics are known: *trapping* outbreaks associated with water rat and muskrat trapping; *agricultural* outbreaks associated with thrashing stacks inhabited by mice rodents; *water-borne* outbreaks due to diseases caused by drinking infected water; *alimentary* outbreaks due to the use of contaminated foodstuffs; *transmissive* outbreaks spread by the bites of bloodsuckers (ticks, stable-flies, mosquitoes, etc.).

Immunity. Following recovery, a stable immunity of long duration develops, being of the tissue and humoral type.

Laboratory diagnosis. The differential diagnosis of plague, anthrax, enteric fever, typhus fever, influenza, malaria, and brucellosis is difficult because these diseases have common symptoms. For differentiation of tularaemia from other diseases laboratory tests are the most effective. Those peculiarities of the disease which can be revealed easily and quickly by laboratory methods are taken into account.

1. Allergy develops on the third-fifth day of the disease. For this reason, intracutaneous and cutaneous tests with tularine are made for early diagnosis. Usually 0.1 ml (10 million dead bacterial cells) is injected intracutaneously, or one drop (50 million) is rubbed into the skin. In tularaemia patients the test gives a positive reaction 6-12 hours after inoculation of tularine (Fig. 85). In distinguishing tularaemia from other infections one must bear in mind that

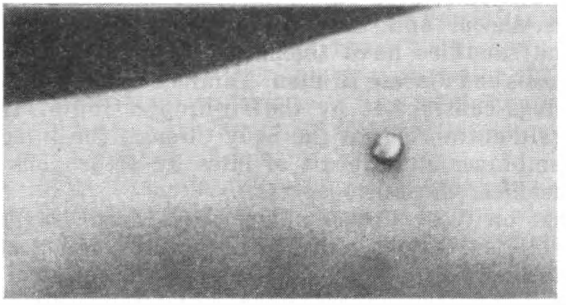


Fig. 85. A positive intracutaneous test for tularaemia

allergic tests may show positive reactions in convalescents and vaccinated individuals.

2. In the second week of the disease agglutinins begin to accumulate in the blood. They are detected by carrying out the agglutination reaction by the blood-drop and volume methods. In some cases this test may give a positive reaction with material containing brucella organisms, since they possess antigens common to tularaemia bacteria.

3. The tularaemia culture is isolated by the biological method as it is impossible to recover the pathogen directly from a tularaemia patient. For this purpose white mice or guinea pigs are infected by material obtained from people suffering from the disease (bubo punctate, scrapings from ulcers, conjunctival discharge, throat films, sputum, and blood). Biological tests are conducted in special laboratories where a standard regimen is observed. The laboratory animals die in 4-12 days if tularaemia bacteria are present in the test material. Autopsy is performed, smears from organs are made and organ specimens are inoculated onto coagulated egg medium for culture isolation. Microscopic, microbiological, and biological studies of the cultured organisms are made. If no culture can be isolated from the first infected guinea pig, an emulsion, obtained from the latter's organs, is inoculated into a second guinea pig, etc.

4. Laboratory diagnosis of rodent tularaemia is made by microscopy of smears from organs, precipitin ring reaction (thermoprecipitation), and biological tests.

Water, foodstuffs, and blood-sucking arthropods are examined by biological tests.

Treatment. Streptomycin, chlortetracycline, tetracycline, oxytetracycline, levomycetin, synthomycin, and a vaccine prepared from killed tularaemia bacteria are prescribed.

Prophylaxis comprises the following:

(1) systematic observation, absolute and relative registration of rodent invasion, and extermination of rats;

(2) prevention of mass reproduction of the rodents;

(3) protective measures in agricultural enterprises against contamination by tularaemia-infected rodents;

(4) protection of foodstuffs and water from rodents;

(5) control of ticks, horseflies, stable-flies, mosquitoes, and protection from these insects;

(6) specific prophylaxis with a live vaccine.

The vaccine is prepared in a dry form. A single application is made by rubbing it into the skin and it produces immunity for a period of 3-6 years. The effectiveness of the tularaemia vaccine is not lower than that of the smallpox vaccine which is considered to be the best.

Over the past years investigations have been conducted in the USSR and some other countries with the aim of improving the existing vaccines. In particular, work is carried out to produce a chemical vaccine which may convey immunity to the air route of infection.

BRUCELLA

In 1886 on the Island of Malta D. Bruce, an English bacteriologist, demonstrated the presence of the causative agent of Malta fever in the spleen of a deceased patient and in 1887 isolated the organism in pure culture.

In 1896 the Danish scientist B. Bang established the aetiology of contagious abortion of cattle. In 1914 the American investigator G. Traum isolated from pigs the organism responsible for contagious abortion among these animals.

A more detailed study of these organisms was made in 1918 by the American scientist A. Evans. She came to the conclusion that, according to their main features, they were all closely related. She grouped them in one genus which she named after D. Bruce, the discoverer of the causative agent of brucellosis. All the former names of the disease (Malta fever, Mediterranean fever, undulant fever, Bang's disease, contagious abortion of pigs, etc.) were substituted by the general name of brucellosis. Brucellae belong to the family *Brucellaceae*.

Morphology. Brucellae are small, coccal, ovoid-shaped microorganisms, 0.3-0.4 μ in size (Fig. 86). Elongated forms are 0.4-3 μ in length and 0.4 μ in breadth. Under the electron microscope *Brucella*

organisms of cattle, sheep and goats appear as coccal and cocco-bacillary forms, while those of pigs are rod-shaped. They are Gram-negative (See Fig. 117, *1*), nonmotile, and do not form spores or capsules (in some strains capsules are sometimes present).

Cultivation. The organisms are aerobic. When cultivated from material recovered from patients, they grow slowly, over a period of 8-15 days; in some cases, however, this period is reduced to 3 days, while in others it is prolonged to 30 days. In laboratory subcultures growth becomes visible in 24-48 hours. The pH of medium for these organisms is 6.8-7.2. The optimal temperature for growth is 37°C, the limits of growth temperatures being 6 and 45°C.

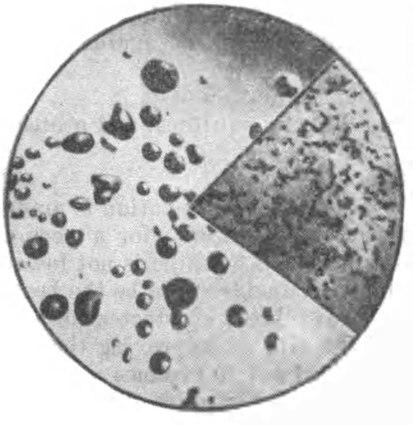


Fig. 86. *Brucella melitensis*, a pure culture and colonies

Brucella organisms may be cultivated on ordinary media, but they grow best on liver-extract agar and liver-extract broth. On liver-extract agar the organisms form round, smooth colonies with a white or pearly hue. In liver-extract broth they produce a turbidity, and subsequently a mucilaginous precipitate settles at the bottom of the tubes. *Brucella* organisms grow well on unfertilized eggs and on the yolk sac of a 10-12-day-old chick embryo.

The brucellae of bovine origin (*Brucella abortus*) only grow in an atmosphere of 10 per cent carbon dioxide, which serves as a growth factor.

Dissociation of the organisms from the S-form to the R-form has been demonstrated. L-forms have also been observed. These forms possess manifest adaptability. They adapt relatively easily to cultivation on nutrient media on which the first generation shows no growth; e. g., the first generation of brucellae of bovine cattle grow well only in the presence of carbon dioxide, while in subcultures they grow without it.

Prolonged cultivation of the organisms on nutrient media leads to a significant weakening of their virulence and to the loss of the Vi-antigen.

Fermentative properties. Brucellae do not liquefy gelatin and do not produce indole. Some strains produce hydrogen sulphide, break down urea and asparagin, reduce nitrates to nitrites, and hydrolize proteins, peptones and amino acids, with release of ammonia and hydrogen sulphide. No carbohydrates are fermented, although a small number of strains ferment glucose and arabinose.

Toxin production. Brucellae do not produce soluble toxins. An endotoxin is produced as a result of disintegration of the bacterial cells. This endotoxin possesses characteristic properties and may be used in allergic skin tests.

Antigenic structure. The organism contains four antigens: A, M, E, and R. The M-antigen is predominant among brucellae of sheep and goats, and the A-antigen, in the other species. Substances of polysaccharide character, with no type specificity, have been extracted from brucellae of cattle, sheep and goats. At present it is known that all three *Brucella* species contain 7 antigens (1, 2, 3, 4, 5, 6, and 7) which are arranged in the form of a mosaic on the cell surface. Brucellae have an antigen common with tularaemia bacteria.

In recent years it has been discovered that brucellae in addition to the O-antigen, possess a thermolabile Vi-antigen. Experiments have confirmed that separate immunization with Vi- and O-antigens does not produce complete immunity to the disease in animals, while simultaneous immunization with all the antigens gives good results.

Classification. Brucellae are divided into three species: (1) brucella of sheep and goats (*Brucella melitensis*); (2) brucella of cattle (*Brucella abortus*); (3) brucella of pigs (*Brucella suis*). All three organisms produce cross immunity. The organisms are differentiated by the characteristics shown in Table 17.

Table 17

Differential Characteristics of *Brucella*

Species of *Brucella*	Conditions of cultivation	Bacteriostatic action of stains				Hydrogen sulphide production
		Fuchsin 1:25,000	Thionin 1:50,000	Methyl violet 1:100,000	Pyronin 1:200,000	
Brucella melitensis	Aerobic	+	+	+	+	—
Brucella abortus	In an atmosphere of 5-10% CO_2	+	—	+	+	+
Brucella suis	Aerobic	—	+	—	—	+

where: + indicates growth and hydrogen sulphide production;
— indicates absence of growth and hydrogen sulphide production.

Types I, II and III have been identified in two species of the organisms (*Br. abortus* and *Br. suis*). They seem to be variants of these species, which have completely or partly lost their virulence.

Brucellae can also be differentiated by the agglutinin adsorption method. This method defines the species of the organisms as well as their genetic interrelationships.

When identifying *Brucella* organisms, the variations in their antigen structure should be considered. Variants that cannot be identified by the usual methods are differentiated by Vi-agglutinating sera and *Brucella* bacteriophage.

Resistance. Brucellae are characterized by high resistance and viability. They survive for a long time at low temperatures. The organisms live for 18 weeks in winter soil, urine, animal faeces, manure, hay dust and bran, up to 16 weeks in ice, snow, butter and sheep's milk cheese, from 12 to 16 weeks in sheep's wool, for 30 days in dust, for 20 days in meat, and for 7 days in milk.

The organisms are sensitive to high temperatures and disinfectants. At 60°C they are destroyed in 30 minutes, at 70°, in 10 minutes, at 80-95°, in 5 minutes, and at boiling point, in a few seconds.

They are very sensitive to phenol, creolin, formalin, chloramine, and other disinfectants. The best results are obtained with a 1 per cent solution of hydrochloric acid in combination with an 8 per cent sodium chloride solution.

Pathogenicity for animals. Goats, sheep, cattle, pigs, horses, camels, deer, dogs, cats, and rodents (rats, mice, susliks, hamsters, rabbits, field-voles, water rats, and other animals) are all susceptible to infection by brucellae.

The disease assumes an acute or a clinically latent course in animals. In sheep and goats abortions and delivery of nonviable fetus are the most typical symptoms. Affected cows have miscarriages, yield less milk, and lose flesh. In the weakest and emaciated animals the disease is sometimes fatal. Apart from miscarriages the organism produces arthritis, bursitis, orchitis, epididymytis, and other complications in pigs. In horses and camels the disease is usually of a latent form, abortions are rare, but emaciation, lassitude and numerous abscesses of long duration occur.

The excretions of sick animals (urine, faeces, amniotic fluid, and uterine mucus), and their milk, particularly that of goats and sheep, and milk products are sources of infection.

The migration of *Brucella* organisms from the usual hosts to animals of other species has been shown. This fact is of great epidemiological importance and must be taken into account in the laboratory diagnosis of brucellosis and when prophylactic measures are applied. Cows infected with *Br. melitensis* may transmit virulent types of *Brucella* organisms to humans.

Among experimental animals guinea pigs are susceptible to brucellosis. The disease lasts for 3 months and the animals die showing lesions in the bones, joints, cartilages and eyes. During the disease they become emaciated, their skin atrophies, the hair falls out, and

orchitis develops. In mice the disease produces septicaemia and the bacteria are recovered from the liver and spleen.

Pathogenesis and disease in man. Brucellosis is a zoonotic infection. Man contracts it from animals (goats, sheep, cows, pigs). From the epidemiological standpoint goats and sheep are the most important. At present there is a sufficient amount of cases to prove that healthy individuals may acquire the disease from humans infected by *Br. melitensis.*

Man contracts brucellosis very often through the milk of goats, sheep, and cows, and dairy products made from such milk also carry infection. The organism may likewise gain entry through abrasions in the skin and mucous membranes. For the most part veterinary and zootechnical staff, shepherds, workers of dairy farms and farms where sheep's milk cheese is produced, workers of stockyards, etc., are attacked by the disease. On farms brucellosis prevails in certain seasons when sheep and goats bear their young (March-May).

From the site of primary location brucellae enter the lymphatic apparatus where they multiply. Then they enter the blood and cause protracted bacteraemia (from 4 to 12 months or longer). Via the bloodstream the bacteria invade the whole body and give rise to orchitis, ostitis, periostitis, arthritis, etc.

Allergy, which develops from the onset of the disease, lasts throughout the disease and remains long after recovery, is characteristic of brucellosis in humans and animals. A sensitized body becomes extremely sensitive to exposure to the specific action of the brucellosis antigen and to various nonspecific factors, such as cooling, secondary infection, trauma, etc.

In human beings brucellosis is characterized by undulant fever with atypical and polymorphous symptoms. The disease may assume an acute septic or a chronic metastatic course. The structural and motor systems, haemopoietic, hepatolienal, nervous and genital systems are often involved. Pregnant women may have miscarriages. Often brucellosis recurs, continuing for months and years. The death rate is 1-3 per cent. The diagnosis of mild, asymptomatic forms presents difficulties and is based on laboratory tests.

Brucellosis in humans has many clinical symptoms common to other diseases (malaria, tuberculosis, rheumatic fever, enteric fever, typhus fever, Q fever, and various septic processes of other aetiology). For this reason, the differential diagnosis of brucellosis is of great importance. It is made with regard to the peculiarities of the disease and of other infections which have a similar course.

Immunity. A characteristic immunity is acquired following brucellosis, the patient becoming insusceptible to repeated infection. Infection and postinfection immunity is of the phagocytic type. At the onset the immunity is nonsterile and infectious, but later it becomes sterile, although labile and of a low grade. All *Brucella* species produce cross immunity in the body.

The humoral factors, i. e., the production of opsonins, agglutinins, complement-fixing substances, and incomplete antibodies which block the causative agents of brucellosis, play a definite role in rendering the brucellae harmless. The phage, being a powerful factor in bacterial variation, plays an important role in producing insusceptibility to brucellosis. Nearly all patients show marked improvement with the clinical course of the disease and on recovery a higher titre and lytic activity of the bacteriophage are displayed.

Laboratory diagnosis. The patient's blood and urine (for isolation of the pathogen), serum (for detection of agglutinins), milk and dairy products (for detection of brucellae or agglutinins in milk) are examined. The microbe is isolated in special laboratories.

1. *Culture isolation.* Since brucellosis is often accompanied by bacteraemia, blood is examined during the first days of the disease (preferably when the patient has a high temperature). For this purpose, 5-10 ml of blood is collected and transferred into two or three flasks (2-5 ml per flask) containing 100 ml of liver-extract or ascitic-fluid broth (pH 6.8). The cultures are grown for 3-4 weeks or more. Five to ten per cent of carbon dioxide is introduced into one of the flasks (for growth of the bovine species of the bacteria). Inoculations on agar slants are made every 4-5 days for isolation and identification of the pure culture.

An antiphage serum is introduced into the cultures for neutralization of the phage which inhibits the growth of brucellae. The best results are obtained when the blood is inoculated into the yolk of an unfertilized egg or the yolk sac of a chick embryo. For this, 0.1-0.2 ml of the tested blood diluted in citrate broth in a ratio of 1:3 is introduced into each egg. The infected eggs are placed in an incubation chamber for 5 days, after which 0.3-0.5 ml of their contents is inoculated into the liquid nutrient media. Growth is examined every 2-3 days.

If the blood culture produces a negative result bone-marrow obtained by sternum puncture is inoculated onto solid and liquid media for isolation of myelocultures.

The urine is also examined. It is obtained with a catheter, centrifuged, and 0.1 ml of the precipitate is seeded onto agar plates containing 1:200,000 gentian violet. In some cases faeces, cow's and human milk, and amniotic fluid of sick humans and animals are examined for the presence of *Brucella* organisms.

Brucella cultures may be isolated by the biological method. For this purpose healthy guinea pigs or white mice are injected with 0.5 or 3 ml of the test material. A month later the guinea pigs' blood is tested for agglutinins, the allergic test is carried out, and the pure culture is isolated. White mice are tested bacteriologically every three weeks.

2. *Serological test.* From the tenth-twelfth day of the disease onwards, the agglutinins accumulate in the blood in an amount

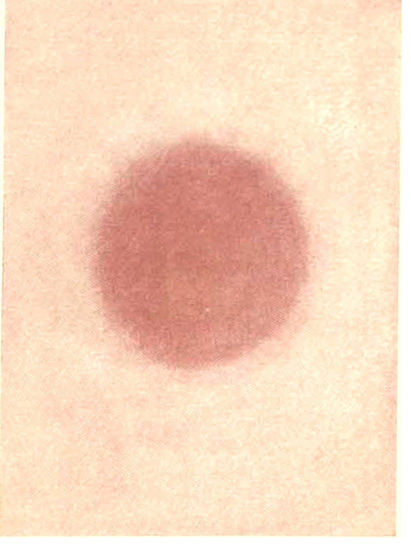


Fig. 87. A positive Burn's allergic test

sufficient for their detection by the agglutination tests. The Wright (in test tubes) and Huddleson (on glass) reactions are carried out. The Wright reaction is valued highly positive in a 1:800 serum dilution, positive, in a 1:400-1:200 dilution, weakly positive, in 1:100 dilution, and doubtful, at a titre of 1:50.

The Huddleson reaction is used mainly in mass examinations for brucellosis.

However, there is a disadvantage of this reaction in that it sometimes shows positive results with sera of healthy individuals who have normal antibodies in their blood.

3. *Skin allergic test.* To determine allergy, Burne's test is made beginning from the fifteenth-twentieth day of the disease. A 0.1 ml sample of the filtrate of a 3- or 4-week-old broth culture (brucellin) is injected intracutaneously into the forearm. The test is considered positive if a painful red swelling 4 by 6 cm in size appears within 24 hours (Fig. 87).

4. *Opsono-phagocytic test.* This test detects changes in the phagocytic reaction. The index of healthy individuals averages 0-1 and occasionally 3-5. In sick persons the reaction is considered high if the index is 50-70, mild, if it is 25-49, and low, if it is 10-24.

For detecting brucellae in the external environment the reaction for demonstrating a rise in bacteriophage titre is carried out.

5. In some cases the *complement-fixation test* is used.

Treatment. Patients suffering from brucellosis are treated with antibiotics (levomycetin, synthomycin, chlortetracycline, streptomycin, dihydrostreptomycin, tetracycline, etc.). Chronic cases are best treated by vaccine therapy, X-ray therapy, blood transfusions, electropyrexia, and balneotherapy.

Prophylaxis comprises a complex of general and specific measures carried out in conjunction with veterinary services. This includes:

(1) early recognition of brucellosis, hospitalization of sick individuals, exposure of the sources of the disease;

(2) sanitary treatment of cattle-breeding farms, identification, examination and isolation of sick animals, immunization with live vaccine;

(3) systematic disinfection of discharges of sick humans and animals, prophylactic disinfection of hands of shepherds and persons engaged in the care of sick animals;

(4) observance of hygienic measures during consumption of milk (pasteurization or boiling) and dairy products in districts where there are cases of brucellosis;

(5) protection of cattle-breeding farms, control of cattle driven from farm to farm, the enforcement of quarantine for new cattle, and isolation of young livestock from sick animals;

(6) sanitary education among the population. Immunization of people with the live vaccine is an additional measure in districts where there are cases of brucellosis.

The brucellosis vaccine is injected subcutaneously in a single 1 ml dose into individuals that show a negative Burne test. For skin inoculation the vaccine is applied like in smallpox vaccination, only that previously the Burne test must be carried out. Revaccination is performed after 10-12 months.

S. Nicolle's statement to the effect that brucellosis is a disease of the future and that its control is ineffective is disproved by data officially released by WHO. In 1951 the number of brucellosis patients registered in all countries of the world totalled 53,711, while in 1956 the corresponding number was 21,241.

CAUSATIVE AGENTS OF GLANDERS AND MELIOIDOSIS

CAUSATIVE AGENT OF GLANDERS

The organism *Actinobacillus mallei* was discovered in 1882 by F. Leoffler and H. Schütz. In 1883 N. Vasilyev was the first to show its presence in man. The microbe belongs to the family *Brucellaceae*.

Morphology. The causative agent of glanders is a slender, straight or slightly curved bacillus, 1.5-4 μ in length and 0.3-0.5 μ in breadth. It is pleomorphous (Fig. 88), and segments are often formed

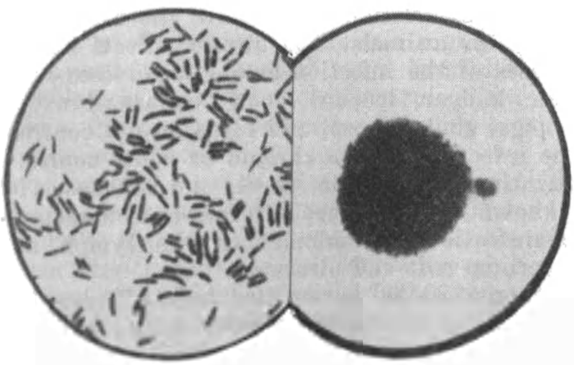


Fig. 88 The glanders bacillus, a pure culture and colonies

in the cells. Thread-like forms, 8-12 μ in length, also occur. There are motile and nonmotile strains. The organisms are Gram-negative and devoid of spores and capsules. They stain best when alkalies or acids are added to the dyes. The short forms show bipolar staining.

Cultivation. The glanders bacteria are aerobes and facultative aerobes. The optimal temperature for growth is 37°C, the limits being 20-45°C. They grow readily on ordinary media but show better growth on coagulated serum or glycerin agar at pH 6.4-6.8. The

colonies have a mucilaginous consistency, are greyish-white in colour, slightly transparent, and have a moist surface (see Fig. 88). On broth growth produces opacity or a precipitate at the bottom of the test tube. The organism grows luxuriantly, on potato medium forming a transparent, honey-like pellicle, amber-brown in colour.

Fermentative properties. The glanders bacteria do not possess great biochemical activity. They do not liquefy gelatin, produce no indole, and do not reduce nitrates to nitrites. They sometimes ferment glucose, with acid formation, and coagulate milk in 4-11 days.

Toxin production. No soluble toxin is produced. The organisms contain an endotoxin. On disintegration they produce, among other substances, mallein which possesses high allergic activity and is employed in the same way as tuberculin for diagnosis.

Antigenic structure and classification. There are two varieties or antigenic groups of these bacteria. The first group contains antigens common to both the glanders and melioidosis organisms, while the second antigen is only possessed by the glanders bacterium. In addition, two fractions have been isolated from the glanders bacterium: a specific polysaccharide and a nonspecific nucleoprotein.

Resistance. The glanders organisms live for 2-3 weeks in putrefying matter, can survive desiccation for 14 days and are not resistant to high temperatures and antiseptics. A 2 per cent formalin solution and 5 per cent calcium chloride kill the bacteria in one hour.

Pathogenicity for animals. The disease affects horses, donkeys, and mules. Cases of the infection among carnivores have also been recorded (lion, badger, leopard, snow leopard, lynx, and prairie cat). Cattle, pigs, goats, sheep, and birds do not contract glanders. In horses the infection runs a chronic or acute course. Depending on the localization, pneumonic, nasal, and cutaneous forms of the disease are known. The cutaneous forms are characterized by the formation of infectious granulomas in the lymph nodes. These granulomas become soft and ulcerated.

In Russia about 250,000 horses died from glanders between 1880 and 1910, and more than 100,000 were killed between 1908 and 1912; over 20,000 animals used to be exterminated annually. In the USSR only isolated cases of the disease are recorded among horses.

Among laboratory animals guinea pigs, cats, and grey mice are susceptible to glanders. Infection of guinea pigs produces swelling, softening, and disintegration of tissues at the site of injection and ulcers and nodules in the internal organs.

Two-three days after an intraperitoneal infection of male guinea pigs a suppurative inflammation of the membranes of the testicles develops with swelling and redness of the skin (Strauss phenomenon). Infection of grey mice produces septicaemia.

Pathogenesis and disease in man. The main sources of infection are animals suffering from glanders. The disease is acquired through

contact with animals and their excretions and from contaminated harnesses, buckets, sacks, forage and water. The disease is contracted more often by stable-men, coachmen, veterinary and laboratory workers.

Glanders is a zoonosis. The disease in humans may be acute or chronic. The pathogen enters the body through skin abrasions, nasal mucosa, mouth, and eyes as well as on inspiration through the upper respiratory tract.

During an acute form of the infection a swelling appears at the site of entry of the glanders bacillus and, later, a nodule develops which disintegrates and ulcerates. This is followed by inflammation of the regional lymph nodes, pustular eruptions on the skin and mucous membranes, and abscesses in the muscles or subcutaneous tissue. The joints, nasal mucosa, and face are sometimes affected. The body temperature is high and there is general lassitude.

In some cases the disease terminates in septicaemia. Autopsy reveals purulent centres in the lungs, spleen, liver, bone marrow, and salivary glands. In Russia in the prerevolutionary period the death rate among people suffering from glanders reached 100 per cent, and averaged 69-86 per cent.

Local granulomas with ulcers develop in chronic forms, characterized by uneven and infiltrated edges. The disease lasts for several months and is accompanied by relapses. Only 50 per cent of the patients recover. Glanders is accompanied by pronounced allergy.

Immunity. In its first stage, immunity acquired in glanders is infectious (nonsterile). The second stage terminates in complete clinical and biological recovery and production of sterile immunity. Agglutinins, precipitins, and complement-fixing antibodies appear in human and animal blood.

Laboratory diagnosis consists of the following procedures:

(1) microscopic examination of material collected from ulcers in the nasal cavity and by puncture of lymph nodes and abscesses. Detection of bacteria that are similar to the causative agent of glanders constitutes a preliminary stage of laboratory diagnosis;

(2) inoculation of the pathologic material on coagulated serum or potatoes and inoculation of blood into 3 per cent glycerin broth; isolation of the pure culture, its identification, and its testing on animals. Attempts to isolate the pure culture from patients has very rarely been successful;

(3) inoculation of the test material or isolated culture into male guinea pigs (intraperitoneally) or into grey mice or cats (subcutaneously in the occipital region). This is the most precise method of glanders diagnosis;

(4) complement-fixation reaction (principal method of laboratory diagnosis of glanders in animals);

(5) the mallein test for diagnosing glanders in humans is performed similar to Pirquet's test, using mallein diluted in a ratio of

1 in 2 or 1 in 10, or as an intracutaneous test with 0.1 ml of mallein diluted in a ratio of 1 in 1,000.

Treatment. Chlortetracycline in combination with sulphonamides (norsulphazol) and streptomycin are prescribed. Autovaccine therapy is employed in chronic cases.

Prophylaxis. Glanders prevention consists of early identification of the infection among horses. This is achieved by performing the mallein test and serologic reactions. Sick animals are killed. In veterinary practice prophylactic immunization of animals with a special vaccine is carried out.

A sick person is not a dangerous source of glanders. However, isolation of the patient and disinfection of premises are compulsory.

In the USSR no cases of glanders have occurred among humans over the last few years. The disease occurs in African, Asian, and American countries.

CAUSATIVE AGENT OF MELIOIDOSIS

Pseudomonas pseudomallei was discovered in 1912 by A. Whitemore and K. Krishnaswami. It is classed in the family *Pseudomonaceae*, order *Pseudomonadales*.

Morphology. The melioidosis bacterium is motile (lophotrichous), curved, granular, and with rounded ends. It measures 2-8 μ in length and 0.5-1 μ in breadth. The organism does not form spores or capsules and resembles the glanders bacillus. In smears the bacteria occur in pairs. The presence of capsule-like structures is a characteristic property. The organisms stain well by the Romanowsky-Giemsa method as well as with all aniline dyes. They are Gram-negative and display bipolar staining, and in this respect resemble *P. pestis*. With repeated subculture they lose their motility and bipolarity.

Cultivation. The causative agent of melioidosis grows both in aerobic and anaerobic conditions. The optimal temperature for growth is 37°C. It can be cultivated on ordinary media with pH 6.8-7.2. On coagulated serum and glycerin agar the organisms form white, smooth, rich glistening colonies which later become dry and flat. Melioidosis organisms differ from the glanders bacilli by their motility and their ability to liquefy gelatin. Older colonies show marked changes, becoming wrinkled and mucilaginous. Melioidosis bacilli do not possess haemolytic properties.

On 5 per cent glycerin agar dry, wrinkled colonies appear within 48 hours. On meat-peptone agar smooth colonies are formed in 24 hours, becoming rough and flat in 2-3 days, and producing a yellow-brown pigment in 4-7 days. In meat broth a slight turbidity is produced, which later becomes denser. A cream-coloured pigment is produced on potatoes.

Fermentative properties. With a stab inoculation melioidosis bacilli liquefy gelatine, coagulated serum, and egg white within 4-5 days. They do not produce indole or hydrogen sulphide. The organisms reduce nitrates to nitrites and ferment and coagulate milk. They ferment glucose, lactose, saccharose, maltose, mannitol, and dulcitol, with acid production. They also hydrolyze urea. The cultures produce a characteristic aromatic odour.

Toxin production. The causative agent of melioidosis does not produce an exotoxin but yields several fractions of endotoxic substances: a weak thermostable endotoxin which gives rise to an erythema at the site of injection in animals and two virulent thermolabile substances. One of the latter is responsible for haemorrhagic-necrotic conditions, while the other is lethal for animals and produces no marked changes in the skin and tissues at the site of introduction.

Antigenic structure and classification. The organism possesses an H-antigen (flagellated) and an O-antigen. The latter is common to the antigens of glanders bacteria and certain salmonellae and, therefore, is not specific. M- and K-antigens have also been revealed; they are characterized by type-specific serological properties and are agglutinated by the corresponding sera. The M-antigen hinders agglutination with O- and K-sera, and the K-antigen prevents O-serum agglutination.

A specific bacteriophage has been isolated from old cultures of melioidosis bacteria. Two phage types have been differentiated: South Vietnamese and North Vietnamese.

Resistance. The organism may survive in the external environment over a long period of time without losing its virulence. On glycerin agar pathogenicity is preserved for 8 years. Guinea pigs inoculated with the bacilli die in 2-3 weeks. The organism is resistant to desiccation and survives in soil for up to a month. It remains virulent in water for 44 days, in soil, for 30 days, and in animal cadaver, for 8 days. It is quickly destroyed by boiling. A 1 per cent phenol solution or a 0.1 per cent formalin solution kills the bacilli in 24 hours.

Pathogenicity for animals. The melioidosis bacillus is pathogenic for rats, mice, cats, dogs, horses, sheep, goats, and monkeys. Among laboratory animals guinea pigs, rabbits, white rats, and mice are susceptible to the infection. The disease is accompanied by septicaemia and development of numerous abscesses in the lymph nodes, liver, spleen, and lungs. In male guinea pigs orchitis occurs.

Pathogenesis and disease in man. Melioidosis is a zoonosis, its clinical characteristics resembling those of glanders. Up to 1933 95 cases of the disease were recorded throughout the world, 90 of which terminated in death. All the cases occurred in a limited area of the Far East, including the Malay Archipelago and adjacent regions of Indochina, Burma, and India. Later the number of re-

corded cases increased to 300, which included several American soldiers who contracted the disease during the war in Korea. Almost all of the patients died. On the Malay Archipelago, in China, Burma, and India the infection is encountered among rats in which the disease is chronic and ends in death. In nature melioidosis occurs as an epizootic among rodents (wild rats and mice), sometimes among guinea pigs and rabbits in vivariums, and also among cats, dogs, and pigs (when they feed on rodent cadaver).

The causative agent of melioidosis is excreted in the nasal discharge, the purulent-mucous discharge of skin ulcers, sputum, and in faeces of the infected animals. These excretions contaminate the land, living quarters, foodstuffs, and other objects.

Humans acquire the disease by eating food contaminated by rodents. Rat fleas and mosquitoes serve as reservoirs and vectors of the causative agent.

The disease proceeds as a generalized septic infection, being acute, subacute, or chronic. The acute condition is characterized by high temperature, severe headache, dyspnoea, vomiting, diarrhoea, aching in the muscles, and leucocytosis (counts up to 15,000). These symptoms are followed by the appearance of abscesses in the muscles and purulent pustules on the skin. Most patients die within 5-10 days from the onset of the disease.

In subacute melioidosis suppurative processes prevail in various organs and tissues: lung abscesses, suppurative orchitis, myositis, osteomyelitis with the development of numerous fistulas; occasionally meningitis is encountered. The patient dies within 3-4 weeks.

Numerous skin ulcers and fistulas appear in the gluteal region in cases of chronic melioidosis. The disease lasts for several months and ends in death.

The clinical characteristics of acute melioidosis are similar to those of pneumonic and septicaemic forms of plague, acute glanders, enteric fever, and the comatose form of malaria. Chronic melioidosis must be differentiated from tertiary syphilis, tuberculosis of the skin, chronic glanders, brucellosis, and mycosis of the skin and bones.

Immunity has not been sufficiently studied. It is probable that no immunity is acquired in humans or animals following the disease.

Laboratory diagnosis. This is carried out by inoculating blood, pus, and material from cadavers onto nutrient media, isolating the pure culture, and identifying it according to its cultural, fermentative, and biological properties.

Infection of guinea pigs through the mucous membranes results in suppurative conjunctivitis, rhinitis, and vaginitis, with ulcerations, high pyrexia, and enlargement and suppuration of the lymph nodes. Convulsions appear on the sixth-eighth day, and the animals die.

Table 18

Main Differential Characteristics of the Causative Agents of Glanders and Melioidosis

Causative agent	Motility	Staining properties	Growth on glycerin agar	Growth on potatoes	Gelatin liquefaction	Fermentation of			Pathogenicity for	
						glucose	lactose	saccharose	rabbits	rats
Actinobacillus mallei	∓	Staining reveals segmentation	Greyish-white film, opaque and slimy	Amber-brown film resembling a drop of honey	—	—	—	—	Slightly susceptible	Susceptible
Pseudomonas pseudomallei	+	Bipolar staining	Colonies similar to those of mycobacteria	Rich cream-coloured film	+	A	A	A	Susceptible	Highly susceptible

where: A indicates carbohydrate fermentation with acid formation;
+ indicates gelatin liquefaction;
∓ indicates nonmotility or slight motility;
— indicates absence of gelatin liquefaction and carbohydrate fermentation.

A subcutaneous inoculation produces a swelling at the site of injection, necrosis and an ulcer with poorly defined edges develop 2-3 days later. The lymph nodes enlarge, suppurate, and become hard. Pyemic foci appear in the organs. The animals die on the twentieth day after inoculation.

Intraperitoneal infection causes peritonitis, and orchitis in males (Strauss phenomenon); the bacteria localize in the testicular exudate.

Post-mortem examination reveals the presence of caseous nodules in all the organs and enlargement of the liver and spleen. Similar lesions are found in the lungs, kidneys, urinary bladder, gall bladder, subcutaneous tissue, muscles, and bones.

The causative agent of melioidosis grows more rapidly on nutrient media than does the glanders bacterium. The former produces wrinkled colonies in 48 hours. An agglutination reaction with patient's serum in a titre of 1:60 is significant, and in a titre of 1:160 is diagnostic.

The indirect agglutination reaction is more specific. It is performed with human erythrocytes sensitized by extracts of melioidosis bacteria cultures. Factors for the differentiation of glanders bacteria from melioidosis bacteria are given in Table 18.

Treatment. Large doses of sulphonamides are used (norsulphazol, sulphazine). Massive doses of antibiotics (penicillin, levomycetin, chlortetracycline, streptomycin, etc.) are prescribed for suppression of the accompanying microflora which may become pathogenic.

Prophylaxis. Patients are transferred to hospitals. Disinfection, disinfestation, and extermination of rats are carried out at the sources of disease by laying rat poison (krisid), barium carbonate, snares, and traps. Prevention of disease also includes systematic control of endemic foci, timely identification of epizootics, protection of foodstuffs, food storehouses and water supplies from rats and mice, and protection of vivariums from wild rodents.

ENTERIC, TYPHOID, PARATYPHOID, AND DYSENTERY BACTERIA

The family *Enterobacteriaceae* includes six genera and numerous species of microorganisms, some of which are common inhabitants of the animal and human intestines, while others are the causative agents of toxinfections, paratyphoids, enteric fever, and dysentery. They belong to the class *Schizomycetes*, order *Eubacteriales*. These bacteria are related genetically and have undergone considerable evolution over a long period of time.

In regard to interrelations with the macroorganism the bacteria of the genus *Escherichia* are most significant; these organisms are common inhabitants of the large intestine, and are usually saprophytes (commensals), *Escherichia coli* being a typical representative of this genus. The genus *Salmonella* consists of microbes pathogenic for humans and animals, and which are the causative agents of toxinfections, paratyphoids, and enteric fever; and the genus *Shigella* includes the bacteria responsible for dysentery.

The enteric, typhoid, paratyphoid, and dysentery bacteria are characterized by common properties: they occur as rods with rounded ends, 0.3-0.8 μ in breadth and 1.5-4 μ in length, and possess no spores. They are Gram-negative, aerobic or facultatively anaerobic.

They differ in relation to fermentative activity. The coli bacterium differs from the pathogenic species (*Salmonella* of enteric fever, paratyphoids A and B, etc.) by its high biochemical activity.

The bacteria of this group can be differentiated by the following properties: motility, fermentation of carbohydrates, production of indole and hydrogen sulphide, and by their antigenic properties which are determined by serological reactions with known species-specific and type-specific agglutinating sera.

ESCHERICHIA COLI

The organism was isolated from faeces in 1885 by T. Escherich. *E. coli* is a common inhabitant of the large intestine of human beings and mammals. It is also found in the intestines of birds, rep-

tiles, amphibians, and insects. The bacteria are excreted in great numbers with the faeces and are always present in the external environment (soil, water, foodstuffs, and other objects).

Morphology. Morphologically *E. coli* corresponds to the above mentioned characteristics of the family *Enterobacteriaceae* (Fig. 89*a*) but is distinguished by its pleomorphism. It occurs in motile and nonmotile forms.

Cultivation. *E. coli* is an aerobe and facultative aerobe. The optimal temperature for growth is 30-37°C and the optimal pH value of medium is 7.2-7.5. The organism also grows readily on ordinary media at room temperature and at 10 and 45°C, growth becomes

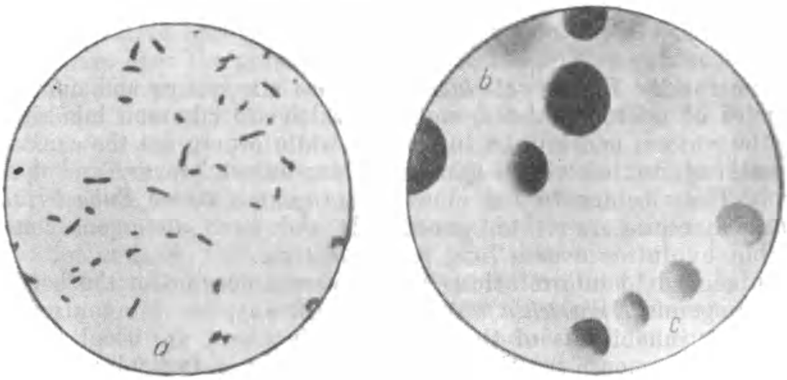


Fig. 89. *a*—smear from *E. coli* culture; *b*—colonies of *E. coli*; *c*—colonies of dysentery bacilli

visible in the first two days. *E. coli* from cold-blooded animals grows at 22-37°C but not at 42-43°C.

On meat-peptone agar *E. coli* produces slightly convex semitransparent, greyish colonies (Fig. 89*b*), and in meat broth it forms diffuse turbidity and a precipitate.

The organism produces colonies which are red on Ploskirev's medium, red with a metallic hue on Endo's medium, and dark-blue on Levin's medium.

Fermentative properties. *E. coli* does not liquefy gelatin. It produces indole and hydrogen sulphide, and reduces nitrates to nitrites. Ferments glucose, levulose, lactose, maltose, mannitol, arabinose, galactose, xylose, rhamnose, and occasionally saccharose, raffinose, dulcitol, salycin, and glycerin, with acid and gas formation. It also coagulates milk.

There are varieties of the bacteria which ferment saccharose, produce no indole, have no flagella, and do not ferment lactose.

Toxin production. Certain strains of *E. coli* are conditionally pathogenic. They contain a gluco-lipo-protein complex with which

their toxic, antigenic, and immunogenic properties are associated. Some strains possess haemolytic properties. Pathogenic cultures possess endotoxins and thermolabile neurotropic exotoxins. The latter accumulate in broth cultures on the second-fourth day of cultivation, while the endotoxins appear only after the twentieth day. Haemotoxins and pyrogenic substances have been obtained from pathogenic strains.

Antigenic structure. The antigenic structure of *E. coli* is characterized by variability and marked individuality. Along with the H- and O-antigens, the presence of other antigens has been shown in some strains, i.e., the surface somatic (membranous, capsular) K-antigen which contains the thermolabile L-, B-, and Vi-antigens and the thermostable A- and M-antigens.

Each antigen group in its turn is composed of a number of antigens designated by Arabic numbers, e.g., the O-group has 137 antigens, the K-subgroup 79, the H-subgroup 40, etc. On the basis of antigenic structure an antigenic formula is derived which fully reflects the antigenic properties of the strain. For example, one of the most widely spread strains is designated O111:B4, etc., or, more fully $\frac{O}{O111}:\frac{K}{B4}:\frac{H}{2}$. Influenced by transformation, lysogenic conversion, transduction, and conjugation (see p. 156), *E. coli* may change its antigenic properties. *E. coli*, harboured by patients with dysentery, enteric fever, or paratyphoids, becomes agglutinable by dysentery, enteric fever, paratyphoid A and B agglutinating sera (see "para-agglutination", p. 240).

Numerous varieties of the organism are produced on cultivation under artificial conditions. Such varieties are not only of theoretical interest, but also of great practical importance in laboratory diagnosis of enteric infections.

Classification. The genus *Escherichia* includes *E. coli*, *E. freundi*, *E. intermedia*, and others. *E. coli* comprises several varieties which are differentiated by their cultural and biochemical properties. F. Kauffmann has detected 25 O-groups responsible for various diseases in humans.

About 50 phage types have been revealed among *E. coli* organisms. They are used in laboratory diagnosis as confirmatory characteristics of the isolated strains.

Resistance. *E. coli* survives in the external environment for months. It is more resistant to physical and chemical factors of the external environment than the typhoid and dysentery bacteria. *E. coli* is killed comparatively rapidly by all methods and preparations used for disinfection. At 55°C the organism perishes in 1 hour, and at 60°C in 15 minutes. *E. coli* is sensitive to brilliant green.

E. coli is used as a test microbe in the assay of disinfectants and methods of disinfection and also in titration of certain antibiotics.

Pathogenicity for animals. The pathogenic strains of *E. coli* cause severe infections in calf sucklings giving rise to an extremely high mortality.

A parenteral injection of the culture into rabbits, guinea pigs, and white mice results in a fatal toxico-septical condition.

Pathogenesis and diseases in man. There are *E. coli* strains pathogenic for human beings. The existence of a number of O-group serotypes has been shown: 25, 26, 44, 55, 75, 86, 111, 112, 114, 119, 125, 127, 128, 145, etc., which are responsible for colienteritis in children.

Colienteritis begins acutely with high temperature (38-39°C), and frequently with severe meteorism, vomiting, diarrhoea, and general toxicosis. The disease usually occurs in children during the first year of life.

The infection is acquired from sick children or carriers. Pathogenic *E. coli* strains are found on various objects. It is assumed that colienteritis is transmitted not only by the normal route for enteric infections but also through the respiratory tract by the droplets and dust.

The pathogenesis of colienteritis depends entirely on the condition of the body. In prematurely born infants and in infants during the first months of life the bactericidal activity of blood is considerably lower in respect to the pathogenic *E. coli* serotypes in comparison to the nonpathogenic types. The reactivity of the child's body at the time of infection plays an important role in the mechanism of resistance to the pathogenic strains. The pathological process develops mainly in the small intestine. Most probably, the mucous membrane of the small intestine in particular is exposed to the action of thermolabile toxic substances.

E. coli may be the cause of colibacillosis in adults (peritonitis, meningitis, enteritis, toxinfections, cystitis, pyelitis, pyelonephritis, angiocholitis, salpingo-oophoritis, appendicitis, otitis, puerperal sepsis, etc.). Overstrain, exhaustion, and conditions following infectious diseases facilitate the onset of various *E. coli* infections. In a number of cases the organism is responsible for food poisoning.

Immunity. Type-specific immunity is acquired following diseases caused by pathogenic strains of *E. coli*. These strains possess a great variety of serotypes which produce no cross immunity, and as a result of this re-infection is possible.

Some varieties of *E. coli* possess properties antagonistic to the pathogenic microbes of the coli group and are used in therapeutic and prophylactic preparations ("mutaflor" strain, *E. coli* M_{17}, etc.). Besides this, *E. coli* as well as other common inhabitants of the intestines are capable of synthesizing various vitamins (K_2, E, and group B) which are indispensable to the human body. The ability of various *E. coli* strains to suppress the growth of *Myco-*

bacterium tuberculosis has also been observed. The suppression of *E. coli* and other members of the biocoenosis may result in a chronic disease known as dysbacteriosis.

Laboratory diagnosis. The patients' faeces, throat and nasal discharges, material obtained at autopsy (blood, bile, liver, spleen, lungs, contents of the small and large intestines, pus), water, foodstuffs, and samples of washings of objects and hands of staff of maternity hospitals, hospitals, and dairy kitchens are all used for laboratory examination during colienteritis. If possible, faeces should be plated immediately after it has been collected. The throat and nasal discharges are collected with a sterile swab. Specimens of organs obtained at autopsy are placed in separate sterile jars.

The tested material is inoculated onto solid nutrient media (Endo's, Levin's) and, simultaneously, onto Ploskirev's media and bismuth-sulphite agar for isolation of bacteria of the typhoid-paratyphoid and dysentery group. Blood is first inoculated into broth and then subcultured on solid media when development of a septic process is suspected. Pus is collected for examination in suppurative lesions. It is placed into a dry sterile vessel and then inoculated onto the differential media of Endo or Levin. The pure culture isolate is identified by its morphological, cultural, biochemical, serological, and biological properties.

Treatment. Patients with colienteritis are prescribed antibiotics (tetracycline with vitamins C, B_1, and B_2, neomycin, chlortetracycline, oxytetracycline, synthomycin, and levomycetin) and biopreparations (coliautovaccine, colibacteriophage, colicin, sour-milk products according to Metchnikoff, and Perets' preparations). Injections of physiological solutions with glucose are administered as measures for controlling toxicosis.

Prophylaxis. In the prevention of diseases caused by pathogenic serotypes of *E. coli*, special attention is given toward early identification of individuals suffering from colienteritis, and also to their hospitalization and effective treatment. There is a necessity for regular examination of personnel in children's institutions as well as of mothers whose children are suffering from dyspepsia. Considerable importance is assigned to observation of sanitary regulations in children's institutions, infant-feeding centres, maternity hospitals, and children's nurseries. Protection of water and foodstuffs from contamination with faeces, the control of flies, and the gradual improvement of standards of hygiene of the population are also particularly important.

Sanitary significance of *E. coli*. This organism is widely spread in nature. It occurs in soil, water, foodstuffs, and on various objects. For this reason *E. coli* serves as an indicator of faecal contamination of the external environment.

Detection of *E. coli* is of great importance in estimating the sanitary index of faecal contamination of water, foodstuffs, soil, bev-

erages, objects, and hand-washings. The degree of contamination of water, soil and foodstuffs is determined by the coli titre or coli index (these terms have been discussed in the chapter concerning the spread of microbes in nature, p. 109). Faecal contamination of articles of use is estimated by qualitative determination of the presence of *E. coli.*

ENTERIC FEVER AND PARATYPHOID SALMONELLAE

The causative agent of enteric (typhoid) fever, *Salmonella typhosa*, was discovered in 1880 by K. Eberth and isolated in pure culture in 1884 by G. Gaffky. A haemoculture was obtained in 1887 by A. Viltchur.

In 1896 the French scientists C. Archard and R. Bensaude isolated paratyphoid B bacteria from urine and pus collected from patients with clinical symptoms of typhoid fever. The bacterium responsible for paratyphoid A (*Salmonella paratyphi*) was studied in detail in 1902 by the German bacteriologists A. Brion and H. Kayser, and the causative agent of paratyphoid B (*Salmonella schottmuelleri*) was studied in 1900 by H. Schottmuller.

Morphology. The morphology of the typhoid salmonella corresponds with the general characteristics of the *Enterobacteriaceae* family, described on p. 341 (Fig. 90*a*). The majority of the strains

Fig. 90. *Salmonella typhosa*
a—smear; *b*—cell with flagella; *c*—colonies

is motile and possesses flagella, from 8 to 20 in number. It is possible that the flagella form various numbers of bunches (Fig. 90*b*).

The paratyphoid salmonellae do not differ from the typhoid organisms in shape, size, type of flagella, and staining properties.

The typhoid salmonellae possess individual and intraspecific variability. When subjected to disinfectants, irradiation, and to the action of other factors of the external environment they change size and shape. They may become coccal, elongated (8-10 μ), or even threadlike.

Cultivation. The typhoid and paratyphoid organisms are aerobes and facultative aerobes. The optimum temperature for growth is 37°C, but they also grow at temperatures between 25 and 40°C. They grow on ordinary media at pH 6.8-7.2. On meat-peptone agar *Salm. typhosa* forms semitransparent fragile colonies which are half or one-third the size of *E. coli* colonies (Fig. 90*c*). On gelatin the colonies resemble a grape leaf in shape. Cultures on agar slants form a moist transparent film of growth without a pigment and in meat broth they produce a uniform turbidity.

On Ploskirev's and Endo's media *Salm. typhosa* and *Salm. paratyphi* form semitransparent, colourless or pale-pink coloured colonies. On Levin's medium containing eosin and methylene blue the colonies are transparent and bluish in colour, on Drigalski's medium with litmus they are semitransparent and light blue, and on bismuth-sulphite agar they are glistening and black.

The colonies produced by *Salm. paratyphi* A on nutrient media (Ploskirev's, Endo's, etc.) are similar to those of *Salm. typhosa* (Fig. 91,*1*).

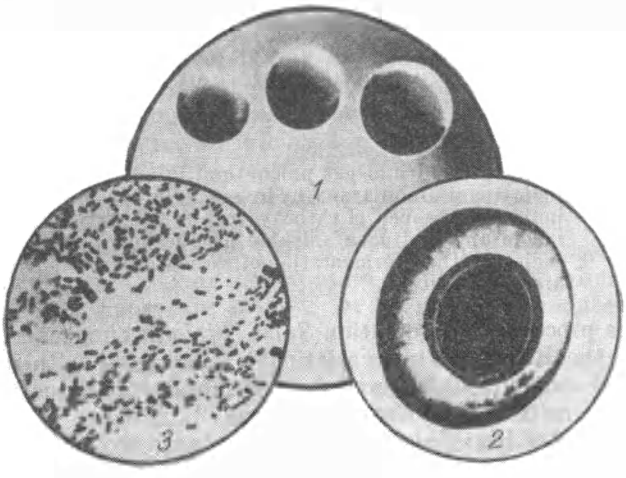


Fig. 91. Colonies of *Salmonella paratyphi* (*1*); colony of *Salmonella schottmuelleri* (*2*); *Salmonella enteritidis* (*3*)

Colonies of *Salm. paratyphi* B have a rougher appearance and after they have been incubated for 24 hours and then left standing at room temperature for several days, mucous swellings appear at their edges (Fig. 91,*2*). This is a characteristic differential cultural property.

Fermentative properties. *Salm. typhosa* does not liquefy gelatin, nor does it produce indole. It produces hydrogen sulphide, and

reduces nitrates to nitrites. The organisms do not coagulate milk, but they give rise to a slightly pink coloration in litmus milk and cause no changes in Rotberger's medium.

They ferment glucose, mannitol, maltose, levulose, galactose, raffinose, dextrin, glycerin, sorbitol and, sometimes, xylose, with acid formation.

Salm. typhosa ferments carbohydrates, with acid and gas formation, and is also distinguished by other properties (Table 19). Two types of *Salm. typhosa* occur in nature: xylose-positive and xylose-negative.

Table 19

Differentiation Between *Salm. typhosa, Salm. paratyphi,* and *E. coli*

Species	Fermentation of carbohydrates					Production of		
	lactose	glucose	maltose	mannitol	saccharose	indole	hydrogen sulphide	ammonia
Salm. typhosa	—	A	A	A	—	—	+	—
Salm. paratyphi	—	AG	AG	AG	—	—	—	—
Salm. schottmuelleri	—	AG	AG	AG	—	—	+	+
E. coli	AG	AG	AG	AG	±	±	+	+

where: A indicates acid production;
AG indicates acid and gas production;
+ indicates ammonia and hydrogen sulphide production;
— indicates absence of carbohydrate fermentation and absence of indole and hydrogen sulphide production;
± indicates that fermentation of saccharose and indole production are not constant.

In the process of dissociation *Salm. typhosa* changes from the S-form to the R-form. This variation is associated with loss of the somatic O-antigen (which is of most immunogenic value) and, quite frequently, with loss of the Vi-antigen.

Toxin production. *Salm. typhosa* contains gluco-lipo-protein complexes. The endotoxin is obtained by extracting the bacterial emulsion with trichloroacetic acid. This endotoxin is thermostable, surviving a temperature of 120°C for 30 minutes, and is characterized by a highly specific precipitin reaction and pronounced toxic and antigenic properties. Investigations have shown the presence of exotoxic substances in *Salm. typhosa* which are inactivated by light, air, and heat (80°C), as well as an enterotropic toxin and pyrogenic substances.

Antigenic structure. *Salm. typhosa* possesses a flagellar H-antigen and thermostable somatic O- and Vi-antigens. All three antigens give rise to the production of specific antibodies in the body, i.e., H-, O-, and Vi-agglutinins. H-agglutinins bring about a large-

flocculent agglutination, while O- and Vi-agglutinins produce fine-granular agglutination.

The antigens differ in their sensitivity to chemical substances. The O-antigen is destroyed by formalin but is unaffected by exposure to weak phenol solutions. The H-antigen, on the contrary, withstands formalin but is destroyed by phenol.

Salm. typhosa, grown on agar containing phenol in a ratio of 1:1,000, loses the H-antigen after several subcultures. This antigen is also destroyed on exposure to alcohol. These methods are employed to obtain the O-antigen in its pure form. The H-antigen is isolated by treating the bacterial emulsion with formalin or by using a broth culture which contains a large number of flagellar components. Immunization with H- and O-antigens is employed for obtaining the corresponding agglutinating sera.

The discovery of the Vi-antigen isolated from virulent *Salm. typhosa* is of great theoretical interest and practical importance.

Vi- and O-antigens are located within the microorganism, on the surface of the bacterial cell. It is assumed that the Vi-antigen occurs in isolated areas and is nearer to the surface than the O-antigen. The presence of Vi-antigens hinders agglutination of salmonellae by O-sera, and the loss of the Vi-antigen restores the O-agglutinability. *Salm. typhosa* which contains Vi-antigens is not agglutinated by O-sera. Vi-agglutinating serum is obtained by saturation of *Salm. typhosa* serum of animals inoculated with freshly isolated salmonellae, employing H- and O-antigens.

The Vi-antigen is a labile substance. It disappears from the culture when phenol is added to the medium and also when the temperature is low (20°C) or high (40°C). It is completely destroyed by boiling for 10 minutes and by exposure to phenol. Exposure to formalin and to temperature of 60°C for 30 minutes produces partial changes in the antigen.

Together with H-, O-, and Vi-antigens, other more deeply located antigens have been revealed. The latter are detected during the change transformation of the bacterial cell to the R-form when the superficial O- and Vi-antigens are lost. The deeply located antigens are nonspecific. Later, salmonellae were found to possess an M-mucous antigen (polysaccharide).

It has been ascertained that the Vi-antigen content of cultures varies, some strains possessing a large quantity of this antigen, while others, only a small quantity. F. Kauffmann subdivides all salmonellae containing Vi-antigens into three groups: (1) pure V-forms with a high Vi-antigen content; (2) pure W-forms which contain no Vi-antigens; (3) transitional V-W-forms which possess Vi-antigens and are agglutinated by O-serum.

Classification. The salmonellae of typhoid fever and paratyphoids together with the causative agents of toxinfections have been included in the genus *Salmonella* (named after the bacteriologist D. Sal-

mon) on the basis of their antigenic structure and other properties. At present, about 700 species and types of this genus are known.

F. Kauffmann and P. White classified the typhoid-paratyphoid salmonellae into a number of groups according to antigenic structure and determined 45 somatic O-antigens. For instance, *Salm. typhosa* (group D) contains three different O-antigens—9, 12, and Vi (Table 20). *Salm. paratyphi* A alone constitutes group A, and *Salm. paratyphi* B belongs to group B. F. Andrewes has proved that the flagellar H-antigen is not homogeneous but is composed of two phases: phase 1 is specific and agglutinable by specific serum, phase 2 is nonspecific and agglutinable not only by specific, but also by group sera. Salmonellae which possess two-phase H-antigens are known as diphasic, while those which possess only the specific H-antigen are monophasic.

In addition, dwarf strains have been discovered, as well as varieties which produce a yellow pigment. On the basis of their susceptibility to the Vi-phage, 56 types of *Salm. typhosa* are recognized, 11 types of *Salm. paratyphosi* B, and 7 types of *Salm. paratyphosi* A.

Resistance. Typhoid and paratyphoid A and B salmonellae survive in ice for several months, in soil contaminated with faeces and urine of patients and carriers, for up to 3 months, in butter, cheese, meat and bread, for 1-3 months, in soil, faecal masses, and water, for several weeks, and in vegetables and fruits, for 5-10 days. They remain unaffected by desiccation and live for a long time in dry faeces. Salmonellae survive for only a short time (3-5 days) in polluted water owing to the presence of a large number of saprophytic microbes and substances harmful to pathogenic microorganisms.

Salm. typhosa and *Salm. paratyphi* are susceptible to heat and are destroyed at 56°C in 45-60 minutes, and when exposed to the usual disinfectant solutions of phenol, calcium chloride, and chloramine, perish in several minutes. The presence of active chlorine in water in a dose of 0.5-1 mg per litre provides reliable protection from *Salm. typhosa* and *Salm. paratyphi*.

Pathogenicity for animals. Animals do not naturally acquire typhoid fever and paratyphoids. Therefore, these diseases are anthroponoses. A parenteral injection of the *Salmonellae* organisms into animals results in septicaemia and intoxication, while peroral infection produces no disease. E. Metchnikoff and A. Bezredka produced a disease similar to human typhoid fever by enteral infection in apes (chimpanzee).

Pathogenesis and diseases in man. The causative agent is primarily located in the intestinal tract. Infection takes place through the mouth.

The pathogenesis of typhoid fever and paratyphoids is characterized by cyclic recurrences and development of certain pathophysiological changes.

Table 20

Serological Classification of Bacteria of the Genus *Salmonella*

Group and species (type)	Antigenic structure		
	somatic antigen	flagellar antigen	
		phase 1	phase 2
Group A			
S. paratyphi A	1, 2, 12	a	—
Group B			
S. schottmuelleri	1, 4, 5, 12	b	1, 2
S. abony	1, 4, 5, 12	b	e, n, x
S. typhimurium	1, 4, 5, 12	i	1, 2
S. stanley	4, 5, 12	d	1, 2
S. heidelberg	4, 5, 12	r	1, 2
S. abortivoequina	4, 12	—	e, n, x
S. abortusovis	4, 12	c	1, 6
S. abortusbovis	1, 4, 12, 27	b	e, n, x
Group C (1, 2)			
S. hirschfeldii	6, 7, Vi	c	1, 5
S. cholerae-suis	6, 7	c	1, 5
S. typhi-suis	6, 7	c	1, 5
S. thomson	6, 7	k	1, 5
S. duesseldorf	6, 8	z_4, z_{24}	—
S. newport	6, 8	e, h	1, 2
S. albany	(8), 20	z_4, z_{24}	—
Group D			
S. typhosa	9, 12, Vi	d	—
S. enteritidis	1, 9, 12	g, m	—
S. dublin	1, 9, 12	g, p	—
S. rostock	1, 9, 12	g, p, u	—
S. moscow	9, 12	g, q	—
S. gallinarum and oth.	1, 9, 12		
Group E (1, 3)			
S. london	3, 10	i, v	1, 6
S. anatum	3, 10	e, h	1, 6
S. harrisonburg and oth.	(3), (15), 34	z_{10}	1, 6

There is a certain time interval after the salmonellae penetrate into the intestines, during which inflammatory processes develop in the isolated follicles and Peyer's patches of the lower region of the small intestine.

As a result of deterioration of the defence mechanism of the lymphatic apparatus in the small intestine the organisms enter the blood. Here they are partially destroyed by the bactericidal substances contained in the blood, with endotoxin formation. During bacte-

raemia typhoid salmonellae invade the patient's body, penetrating into the lymph nodes, spleen, bone marrow, liver, and other organs. This period coincides with the early symptoms of the disease and lasts for a week.

During the second week of the disease endotoxins accumulate in Peyer's patches, are absorbed by the blood, and cause intoxication. The general clinical picture of the disease is characterized by status typhosus, disturbances of thermoregulation, activity of the central and vegetative nervous systems, cardiovascular activity, etc.

On the third week of the disease a large number of typhoid bacteria enter the intestine from the bile ducts and Lieberkühn's glands. Some of these bacteria are excreted in the faeces, while others re-enter the Peyer patches and solitary follicles which had been previously sensitized by the salmonellae in the initial stage. This results in the development of hyperergia and ulcerative processes. Lesions are most pronounced in Peyer's patches and solitary follicles and may be followed by perforation of the intestines and peritonitis.

The typhoid-paratyphoid salmonellae together with products of their metabolism induce antibody production and promote phagocytosis. These processes reach their peak on the fifth-sixth week of the disease and eventually lead to recovery from the disease.

Clinical recovery does not coincide with the elimination of the pathogenic bacteria from the body. The majority of convalescents become carriers during the first weeks following recovery, and 3-5 per cent of the cases continue to excrete the organisms for many months and years after the attack and, sometimes, for life. Inflammatory processes in the gall-bladder (cholecystitis) and liver are the main causes of a carrier condition since these organs serve as favourable media for the bacteria, where the latter multiply and live for long periods. Besides this, typhoid-paratyphoid salmonellae may affect the kidneys and urinary bladder, giving rise to pyelitis and cystitis. In such lesions the organisms are excreted in the urine.

In one, two, or three weeks following marked improvement in the patient's condition, relapses may occur as a result of reduced immunobiological activity of the human body and hence a low-grade immunity is produced.

Due to the wide range in the severity of typhoid fever from gravely fatal cases to mild ambulant forms it cannot be differentiated from paratyphoids and other infections by clinical symptoms. Laboratory diagnosis of these diseases is of decisive importance (see Table 19). In recent years typhoid fever has changed from an epidemic to a sporadic infection, being milder in nature and rarely producing complications.

Diseases caused by *Salm. paratyphi* are similar to typhoid fever.

The period of incubation and the duration of the disease are somewhat shorter in paratyphoid infections than in typhoid fever.

Immunity. Immunity acquired after typhoid fever and paratyphoids is relatively stable but relapses and reinfections sometimes occur. Antibiotics, used as therapeutic agents, inhibit the immunogenic activity of the pathogens which change rapidly and lose their O- and Vi-antigens.

Along with humoral and phagocytic defence mechanisms, certain significance in production of immunity in typhoid fever and paratyphoids is assigned to the barrier function of the reticulo-endothelial system and functional immunity which ensures recovery of the impaired body functions.

Laboratory diagnosis. The present laboratory diagnosis of typhoid fever and paratyphoids is based on the pathogenesis of these diseases.

1. *Isolation of haemoculture*. Bacteraemia appears during the first days of the infections. Thus, for culture isolation 10-15 ml of blood (15-20 ml during the second week of the disease and 30-40 ml during the third week) are inoculated into 100, 150, and 200 ml of 10 per cent bile broth, after which cultures are incubated at 37°C and on the second day subcultured onto one of the differential media (Ploskirev's, Endo's, Levin's) or common meat-peptone agar.

The isolated culture is identified by inoculation into a series of differential media (see Table 19) and by the agglutination reaction. The latter is performed by the glass-slide method using monoreceptor sera or by the test-tube method using purified specific sera.

2. *Serological method*. Sufficient number of agglutinins accumulate in the blood on the second week of the disease, and they are detected by the Widal reaction. Diagnostic typhoid and paratyphoid A and B suspensions are employed in this reaction. The fact that individuals, treated with antibiotics, may yield a low titre reaction must be taken into consideration. The reaction is valued positive in patient's serum diluted 1 in 200 and higher.

The Widal reaction may be positive not only in patients but also in those who had suffered the disease in the past and in vaccinated individuals. For this reason diagnostic suspensions of O- and H-antigens are employed in this reaction. The sera of vaccinated people and convalescents contain H-agglutinins for a long time, while the sera of patients contain O-agglutinins at the height of the disease.

In typhoid fever and paratyphoids the agglutination reaction may be sometimes of a group character since the patient's serum contains agglutinins not only to specific but also to group antigens which occur in other bacteria. In such cases the patient's blood must be sampled again in 5-6 days and the Widal reaction repeated. Increase of the agglutinin titre makes laboratory diagnosis easier. In cases when the serum titre shows an equal rise with several antigens, O-, H-, and Vi-agglutinins are detected separately.

The Vi-agglutination reaction is employed for identification of *Salm. typhosa* carriers. This reaction is performed with sera (inactivated at 56°C for 30 minutes and diluted in the ratio of 1:10-1:80) and diagnostic Vi-suspensions. Individuals who give a positive Vi-agglutination reaction are subjected to microbiological examination for isolation of *Salm. typhosa* from the bile, faeces, and urine. The best results are obtained when Vi-haemagglutination is employed.

For quick serological diagnosis of typhoid fever and paratyphoids Noble's agglutination method and agglutination on glass by the Minkevitch-Brumpt method are carried out. In the latter case the bacterial emulsion is agglutinated in a drop of undiluted blood placed on a slide.

3. *A pure culture is isolated from faeces and urine* during the first, second, and third weeks of the disease. The test material is inoculated into bile broth, Muller's medium, Ploskirev's medium, or bismuth sulphite agar.

Isolation and identification of the pure culture are performed in the same way as in blood examination.

Selective media are recommended for isolation of the typhoid-paratyphoid organisms from water, sewage, milk, and faeces of healthy individuals. These media slightly inhibit the growth of pathogenic strains of typhoid-paratyphoid organisms and greatly suppress the growth of saprophytic microflora.

A reaction for the detection of a rise in the phage titre is employed in typhoid fever and paratyphoid diagnosis. This reaction is based on the fact that the specific (indicator) phage multiplies only when it is in contact with homologous salmonellae. An increase in the number of phage corpuscles in the test tube as compared to the control tube is indicative of the presence of organisms homologous to the phage used. This reaction is highly sensitive and specific and permits to reveal the presence of the salmonellae in various substrates in 11-22 hours without the necessity of isolating the organisms in a pure culture. The reaction is valued positive if the increase in the number of corpuscles in the tube containing the test specimen is not less than 5-10 times that in the control tube.

When unagglutinable cultures of the typhoid and paratyphoid organisms are isolated, the agglutination reaction is performed using Vi-sera. If the latter are not available, the tested culture is heated for 30 minutes at 60°C or for 5 minutes at 100°C. The agglutination reaction is carried out with a suspension of this heated culture.

In some cases a bacteriological examination of duodenal juice (in search for carriers), bone marrow, and material obtained from roseolas is conducted.

Phage typing of typho-paratyphoid organisms is sometimes employed. The isolated culture is identified by type-specific O- and

Vi-phages. Sources of typhoid and paratyphoid infections are revealed by this method.

Water is examined for the presence of typho-paratyphoid bacteria by filtering large volumes (2-3 litres) through membrane filters and subsequent inoculation on plates containing bismuth sulphite agar. If the organisms are present, they produce black colonies in 24-48 hours. The reaction of increase in phage titre is carried out simultaneously.

Treatment. Patients with typhoid fever and paratyphoids are prescribed synthomycin, levomycetin, and chlortetracycline. These drugs markedly decrease the severity of the disease and diminish its duration. Great importance is assigned to general nonspecific treatment (dietetic and symptomatic). Treatment must be applied until complete clinical recovery is achieved, and should never be discontinued as soon as the bacteria disappear from the blood, urine, and faeces since this may lead to a relapse.

The eradication of the organisms from salmonellae carriers is a very difficult problem.

Prophylaxis. General measures amount to rendering harmless the sources of infection. This is achieved by timely diagnosis, hospitalization of patients, disinfection of the sources, and identification and treatment of carriers. Of great importance in prevention of typhoid fever and paratyphoids are such measures as disinfection of water, safeguarding water supplies from pollution, systematic and thorough cleaning of inhabited areas, fly control, and protection of foodstuffs and water from flies. Washing of hands before meals and after using the toilet is necessary. Regular examination of personnel in food-processing factories for identification of carriers is also extremely important.

In the presence of epidemiological indications specific prophylaxis of typhoid infections is accomplished by vaccination. Several varieties of vaccines are prepared: typhoid vaccine (monovaccine), typhoid and paratyphoid B vaccine (divaccine), typhoid and paratyphoid A and B vaccine (trivaccine), typhoid, paratyphoid B, and Flexner and Sonne dysentery vaccine (tetravaccine), and typhoid, paratyphoid A and B, and Flexner and Sonne dysentery vaccine (pentavaccine).

Good effects are obtained also with a chemical associated adsorbed vaccine which contains O- and Vi-antigens of typhoid, paratyphoid B, *Shigella flexneri* and *Sonnei* organisms and a concentrated purified and adsorbed tetanus anatoxin. All the antigens in this vaccine were adsorbed on aluminium hydroxide.

An associated dry vaccine which contains antigens of the bacteria of typhoid fever, paratyphoid B, and Flexner and Sonne dysentery is a variety of the tetravalent vaccine. When there are epidemiological indications, all the above-mentioned vaccines are used according to instructions and special directions of the sanitary and epidemiological service.

SALMONELLAE—CAUSATIVE AGENTS OF FOOD TOXINFECTIONS

The genus *Salmonella* comprises many species and types of bacteria which possess properties similar to those of *Salm. paratyphi* B.

In 1885 in America D. Salmon isolated the bacterium *S. choleraesuis*, which was long considered the causative agent of plague in pigs. Later it was shown to be in association with the causative agent of this disease and the cause of human toxinfections.

In 1888 during a large-scale outbreak of toxinfections in Saxony A. Gärtner isolated *S. enteritidis* bacteria from the flesh of a cow which had to be killed, and also from the spleen of a dead person. The organisms proved to be pathogenic for mice, guinea pigs, rabbits, sheep, and goats.

In 1896 in Breslau K. Kensche and in 1898 in Ertrike G. Nobel discovered *S. typhimurium* (*Bacillus Breslau*) in cases of food poisoning and isolated a pure culture of the organism.

It is now known that among the large number of organisms which comprise the salmonella group, about twenty species and types are pathogenic for humans and are the cause of food poisoning (toxinfections).

Morphology. Morphologically salmonella organisms possess the general characteristics of the family *Enterobacteriaceae*, given on p. 341 (Fig. 91, *3*). They are motile and peritrichous.

Cultivation. The organisms are facultative aerobes, the optimum temperature for growth being 37°C. They grow readily on ordinary nutrient media.

Fermentative properties. Salmonellae do not liquefy gelatin and do not produce indole. The majority of species produce hydrogen sulphide and ferment glucose, maltose, and mannitol, with acid and gas formation.

Toxin production. Salmonellae produce no exotoxin. Their ability to produce diseases in animals and humans is associated with an endotoxin which is a gluco-lipo-protein complex and is characterized by its high toxicity.

Antigenic structure. As was mentioned above, all salmonellae are divided into 35 groups according to their serological properties (see Table 20). Thus, according to the Kauffmann-White Scheme, *S. enteritidis* belongs to group D, *S. typhimurium* to group B, and *S. choleraesuis* to group C.

Classification. The organisms are classified according to their antigenic, cultural, and biological properties (see Table 21).

Resistance. Salmonellae are relatively stable to high temperatures (60-75°C), high salt concentrations, and to certain acids. They withstand 8-10 per cent solution of acetic acid for 18 hours, and survive for 75-80 days at room temperature. The endotoxins remain active within large pieces of meat for long periods (even after the

Table 21

Differentiation of the Main Species of Toxinfection Causative Agents and Paratyphoid B Salmonella *(Salmonella schottmuelleri)*

Causative agent	Appearance of mucous swellings	Pathogenicity for mice when inoculated per os	Serological characteristics			
			group	somatic antigens	flagellar antigens	
					phase 1	phase 2
S. typhimurium	—	Pathogenic	B	1, 4, 5, 12	i	1, 2
S. choleraesuis	∓	Pathogenic	C	6, 7	c	1, 5
S. enteritidis	—	Pathogenic	D	1, 9, 12	g, m	—
S. newport	—	Pathogenic	C_2	6, 8	e, h	1, 2
S. anatum	—	Pathogenic	F	3, 10	e, h	1, 7
S. schottmuelleri	+	Nonpathogenic	B	1, 4, 5, 12	b	1, 2

meat has been cooked) as well as in inadequately fried rissoles and other foods.

A characteristic feature of foodstuffs contaminated by salmonellae is that they show no changes which can be detected organoleptically.

Pathogenicity for animals. Salmonellae, the causative agents of toxinfections, are pathogenic microorganisms which may give rise to paratyphoid in calves, typhoid and paratyphoid in newly-born pigs, typhoid in fowls and pullorum disease in chickens, typhoid in mice and rats, and enteritis in adult cattle.

Among laboratory animals, white mice are most susceptible to the organisms (*S. typhimurium*, *S. enteritidis*, *S. choleraesuis*, etc.). Enteral and parenteral inoculations result in septicaemia in these animals.

Pathogenesis and diseases in man. Ingestion of food contaminated by salmonellae is the main cause of disease. Most frequently food poisoning is due to meat prepared from infected animals and waterfowls without observance of culinary regulations. Eggs of infected waterfowls are also sources of infection. Seabirds are frequent salmonellae carriers. Meat may be infected while the animal is alive or after its death.

As distinct from typhoid fever and paratyphoids A and B, salmonellae toxinfections are anthropo-zoonotic diseases. Intoxication develops in a few hours following infection. Masses of microbes ingested with the food are destroyed in the gastrointestinal tract and in the blood. This results in the production of large amounts of endotoxin which, together with the endotoxin entering the body with the ingested food, gives rise to intoxication. Salmonellae are known to be highly infestive. Bacteraemia usually becomes manifest in the first hours after the onset of the disease.

The disease course is characterized by clinical manifestation of

toxinfectional, gastroenteric, and typhoid- and cholera-like symptoms.

Along with typical zoonotic salmonella diseases, there are salmonelloses which occur as a result of infection from sick people and carriers. Such cases are predominant in newborn and prematurely born children, convalescents, and individuals with chronic diseases. In children's institutions, maternity hospitals, somatic departments of pediatric clinics, and among children suffering from dysentery in departments for contagious diseases the main sources of infection are sick children and bacteria carriers. Children suffering from salmonelloses display symptoms of dyspepsia, colitis (enterocolitis), and typhoid fever, and often these conditions are accompanied by septicaemia and bacteraemia. The diseases are of long duration or become chronic and are sometimes erroneously diagnosed as chronic dysentery.

Immunity acquired after salmonellosis is of low grade and short duration. Low titres of agglutinins (from 1:50 to 1:400 and, rarely, up to 1:800) appear in the blood of convalescents during the second week.

Laboratory diagnosis. Specimens of food remains, washings from objects, stools, vomit, lavage water, blood, urine and organs obtained at autopsy are carefully collected and examined systematically. In the beginning, the specimens are inoculated into nutrient media employed for diagnosis of typhoid fever and paratyphoids A and B. Then the cultural, serological, and biological properties of the isolated cultures are examined (Table 21).

In some cases the biological test is performed not only with the cultures, but also with remains of the food which caused the poisoning.

For retrospective diagnosis blood of convalescents is examined for the presence of agglutinins on the eighth-tenth day after the onset of disease. This is performed by the Widal reaction with suspensions of the main diagnostic bacterial species which cause food toxinfections.

Table 21 shows that differential laboratory diagnosis between *S. typhimurium* and *S. schotmuelleri* is particularly difficult since they have group, somatic, and flagellar phase 2 antigens in common. Pathogenicity for white mice and appearance of mucous swellings and daughter colonies on agar serve as differential criteria.

Treatment. Therapeutic measures include antibiotics (synthomycin, levomycetin, chlortetracycline, oxytetracycline and tetracycline). Good effects are also obtained with stomach lavage, injections of glucose and physiological solution, and cardiac drugs.

Prophylaxis of salmonellae toxinfections is ensured by veterinary and sanitary control of cattle, slaughter-houses, meat factories and fish industries, laboratory control of meat intended for sale, and sterilization of meat which otherwise may not be sold. The medical

hygiene service identifies carriers among people working in food factories, catering houses, and other food-processing establishments and controls the sanitary regulations at food enterprises, shops, store-houses, and in catering houses.

In some cases food poisoning may be caused by conditionally pathogenic bacteria (*Proteus morgani*, *Proteus mirabilis*, *Proteus rettgeri*, *Proteus inconstans*, *E. coli*, etc.).

SHIGELLAE

The causative agent of dysentery was discovered in 1891 by A. Grigoryev. In 1898 this organism was studied in detail by K. Shiga in Japan and in 1900-1901 by V. Kruse in Germany (Shiga bacillus).

In 1900 S. Flexner and R. Strong in the Philippines isolated dysentery organisms (Flexner bacillus) which possessed properties different to those of the above-mentioned bacillus.

In 1904 P. Hiss and F. Russel described a dysentery bacterium which became to be known as the Hiss-Russel bacillus. At present this organism is included in the Flexner species.

In 1904 K. Duval, in 1907 V. Kruse et al., and in 1915 K. Sonne recognized a dysentery bacillus which ferments lactose.

In 1917 M. Stutzer in Russia and K. Schmitz in Rumania simultaneously isolated another species of dysentery bacilli (Stutzeri-Schmitzii bacillus).

Later other bacilli causing dysentery were discovered. According to the current International Nomenclature, all dysentery bacilli are grouped together in one genus known as *Shigella*.

Morphology. Morphologically dysentery bacilli correspond to the organisms of the family *Enterobacteriaceae* whose characteristics are given on p. 342 (Fig. 92). Dysentery bacilli have no flagella, and this is one of the differential characters between these organisms and bacteria of the coli-typhoid-paratyphoid group. Some strains of Flexner bacilli are found to possess cilia (see p. 46).

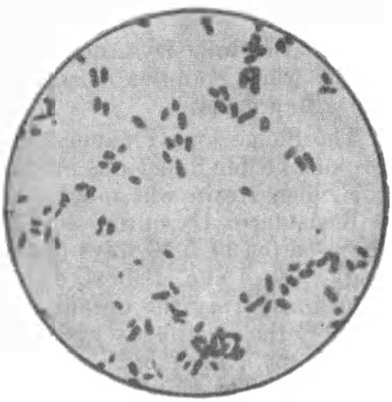


Fig. 92. Flexner's dysentery bacilli

Cultivation. Dysentery bacilli are facultatively aerobic and grow readily on common media at pH 6.7-7.2, the optimal temperature for growth being 37°C,

they do not grow at 45°C. On solid media they form small (1-1.5 mm in diameter), fragile, semitransparent colonies (see Fig. 89*c*) which are similar to those of the typhoid bacteria. In meat broth dysentery bacilli produce a diffuse turbidity.

Fermentative properties. None of the species of dysentery bacilli liquefy gelatin nor produce hydrogen sulphide. They ferment glucose, with acid formation, with the exception of the Newcastle subspecies which sometimes produce both acid and gas during this reaction. With the exception of the Sonne bacilli, none of them ferment lactose.

Toxin production. The Shiga bacillus produces thermolabile exotoxin which displays marked tropism to the nervous system and intestinal mucous membrane. This toxin may be found in old meat broth cultures, lysates of a 24-hour-old agar culture, and in desiccated bacterial cells.

An intravenous injection of small doses of the exotoxin is fatal to rabbits and white mice. Such an injection produces diarrhoea, paralysis of the hind limbs, and collapse.

The dysentery exotoxin causes the production of a corresponding antitoxin. The remaining types of dysentery bacilli produce no soluble toxins. They contain endotoxins, which are of a gluco-lipo-protein nature, and occur in the smooth but not in the rough variants.

Thermolabile substances which produce a neurotropic effect are found in some strains of the Stutzer-Schmitz and Sonne bacilli. They were extracted from old cultures by treating the latter with trichloroacetic acid.

Antigenic structure. Dysentery bacilli are subdivided into 5 species (4 groups) within which serotypes may be distinguished. The antigenic structure of shigellae is associated with somatic O-antigens and surface K-antigens.

Classification. Dysentery bacilli are differentiated on the basis of the whole complex of antigenic (Table 22) and biochemical (Table 23) properties.

The Sonne and Flexner bacilli are the strains which have been responsible for infectious processes in recent years. Up to 1937-1940, the Shiga strain was most common.

Resistance. Dysentery bacilli live in the external environment for a period of 5-10 days (in soil, foodstuffs and water, and on objects, plates and dishes). Direct sunlight and a 1 per cent phenol solution destroy the organisms in 30 minutes and at a temperature of 60°C the organisms perish in 10 minutes. The bacilli are easily killed by treatment with chloramine and calcium chloride solutions. The Shiga bacilli are most sensitive to physical and chemical factors, while the Sonne bacilli are relatively resistant to them.

Table 22

Classification of Shigellae

Soviet					International		
	Species	Subspecies	Type antigen	Sub-type	Subgroup	Type	Sub-type
Do not ferment mannitol	*Sh. shigae*						
	Sh. stutzeri--schmitzii		1	—	A	1	
	Sh. large-sachsi		2	—		2	
			3	—	*Sh. dysenteriae*	3	
			4	—		4	
			5	—		5	
			6	—		6	
			7	—		7	
	Provisional*		8	—		8	
			9	—		9	
			10	—		10	
	Sh. flexneri	*Sh. flexneri*	1	1a	B	1	1a, 1b
				1b	*Sh. flexneri*	2	2a
			2	2a			
				2b		3	2b
			3	3a			3a
				3b		4	3b
				3c			3c
			4	4a			4a
				4b			4b
			5**	5(x+)		5	x+
				5(x—)		6	x—
				var.x^2		x—	
				var.y^2		y—	
Ferment mannitol		*Sh. newcastli*	6	—			
		Sh. boydii	1	—	C	1	
			2	—	*Sh. boydii*	2	
			3	—		3	
			4	—		4	
			5	—		5	
			6	—		6	
			7	—		7	
			8	—		8	
			9	—		9	
			10	—		10	
			11	—		11	
			12	—		12	
			13	—		13	
			14	—		14	
			15	—		15	
Slow fermentation of lactose	*Sh. sonnei*	—	—	—	D *Sh. sonnei*	—	

* Not clear, to which species it is related to.
** Devoid of type antigens and are differentiated by group antigens.

Table 23

Biochemical Properties of Shigellae

Species	Subspecies	Fermentation of carbohydrates					Production of:	
		lactose	glucose	mannitol	maltose	saccharose	indole	catalase
Sh. shigae	—	—	A+	—	—	—	—	—
Sh. stutzeri-schmitzii	—	—	A+	—	—	—	+	±
Sh. large-sachsi	—	—	A+	—	—	—	+	—
Sh. flexneri	*Sh. flexneri*	—	A+	A+	A±	—	+	+
	Sh. newcastli	—	A+ or AG	A±	A±	—	—	+
	Sh. boydii	—	A+	A+	—		±	±
Sh. sonnei		A+ (late)	A+	A+	A+	A+ (late)	—	∓

where: A indicates acid;
AG indicates acid and gas;
± indicates irregular production of catalase;
\+ indicates indole and catalase production;
— indicates no carbohydrate fermentation and no indole and catalase production;
∓ indicates low degree of catalase activity.

Dysentery bacilli may acquire resistance to drugs (sulphonamides, antibiotics) and to ionizing radiation. Variants of the Flexner bacilli have been obtained, which survive exposure even to 1,000,000 r. The growth rate of the radioresistant forms is 33 per cent that of the initial forms.

Pathogenicity for animals. Monkeys are susceptible to dysentery bacilli. They contract the infection from sick people or carriers in the nurseries. In some cases they become sources of contamination of personnel in nurseries and zoological gardens.

Parenteral infection causes fatal intoxication in rabbits. An intravenous injection of a Shiga bacillus culture exerts a highly toxic effect. The resulting infection constitutes diarrhoea, paresis or paralysis of the limbs, followed by collapse and death. Autopsy reveals hyperaemia of the intestinal mucous membrane, haemorrhages, necrosis, and ulcerations. Infected white mice die within the first four days.

When cultures of virulent dysentery bacilli are introduced into the respiratory tract of white mice, the organisms multiply. However, attempts to reproduce dysentery in white mice are of no success. Kittens and puppies are more susceptible. Guinea pigs display low susceptibility to dysentery bacilli, but infection through the eye conjunctiva results in keratoconjunctivitis which is assumed to be a specific lesion.

Pathogenesis and disease in man. People suffering from acute and chronic dysentery as well as carriers are sources of infection. Infection takes place through the mouth by ingestion of contaminated foodstuffs and water, by contaminated hands, house flies, and various objects impregnated with dysentery bacilli. The dysentery organisms are confined to the intestinal mucous membrane and submucous layer where they multiply. The organisms do not invade the blood as a rule. Intoxication is due to absorption of bacillar exo- and endotoxins through the intestinal mucous membrane. Dysentery caused by the Shiga bacillus was of the most severe type. It was accompanied by general intoxication, deep involvement of the intestinal wall—oedema, hyperaemia, and haemorrhagic diarrhoea. The disease is aggravated to a considerable extent by the presence of lamblia and helminths which are fellow members in the parasite coenosis in dysentery.

Inflammatory processes developing in the mucosa of the large intestine produce ulcers. The latter form scars on healing which cause stenosis of the intestinal lumen and patency disturbances.

Immunity. Immunity acquired after dysentery is specific and type-specific but relatively weak and of a short duration. For this reason the disease may recur many times and, in some cases, may become chronic.

Laboratory diagnosis. Reliable results of laboratory examination depend, to a large extent, on correct sampling of stool speci-

mens and its immediate innoculation onto a selective differential medium. The procedure should be carried out at the patient's bedside, and the plate sent to the laboratory.

In hospital conditions the stool is collected on a paper plate or napkin, placed into a bedpan. The latter should be washed previously with running water or, better still, with boiling water, be dry, and should contain no disinfectants. It is best to collect the faeces directly from the rectum by means of a rectal tube or rectal swab. The specimen should be sown in the isolation department immediately after collection. Portions of the stool, containing pus and mucus, are picked out with a swab and plated on Ploskirev's medium. The plates are incubated at 37°C for 24 hours. The isolated pure culture is identified by its biochemical and serological properties.

An accelerated method of dysentery diagnosis is employed to shorten the examination period. In some cases an agglutination reaction, similar to the Widal reaction, is used. This test is relevant to retrospective diagnosis.

Difficulties in identification of atypical inagglutinable cultures are encountered currently. In such cases the agglutination reaction is repeated with a culture previously heated to 100°C for 30 minutes. Agglutinability may be restored by a number of subcultures or subinoculations onto 10-20 per cent bile broth or an agar slant containing mannitol and a BP indicator.

Dysentery bacilli, devoid of a surface antigen, are not agglutinated by specific sera and as a rule do not break down urea. The specific antigen remains deep within the bacterial cell and its presence is detected by a precipitin reaction with the complete antigen.

The nature of the isolated culture may be determined in some cases by its lysis by a polyvalent dysentery phage and by the keratoconjunctival test performed on guinea pigs.

Treatment. Patients are given antibiotics (tetracycline, chlortetracycline, oxytetracycline, levomycetin, and synthomycin) and sulphonamides (sulgine, phthalazole, etc.). Neomycin is recommended in cases when the isolated bacilli are resistant to the commonly used antibiotics. An alcohol vaccine is injected to prevent transition of an acute form of the disease into a chronic one. It should be borne in mind that all infectious diseases, and dysentery in particular, are accompanied by vitamin deficiency. For this reason, a patient with dysentery should be given a high-caloric diet, rich in vitamins. Such a diet will facilitate rapid rehabilitation of the disturbed functions and increase of immunobiological activity.

Prophylaxis. Dysentery control is ensured by a complex of general and specific measures; (1) early and a completely effective clinical, epidemiological, and laboratory diagnosis; (2) hospitalization of patients or their isolation at home with observance of the required regime; (3) thorough disinfection of sources of the

disease; (4) adequate treatment of patients with highly effective antibiotics and use of chemotherapy and immunotherapy; (5) control of disease centres with employment of prophylaxis measures.

Prophylactic vaccination is carried out among people as an auxiliary measure in the presence of epidemiological indications. Usually the tetravalent vaccine containing typhoid, paratyphoid B, and dysentery Flexner and Sonne bacilli is employed. Vaccination against dysentery is of low efficiency. According to epidemiological indications, dysentery phage is also given as a preventive measure.

THE CHOLERA VIBRIO

Cholera dates back to the most ancient times. Its endemic focus is India (Lower Bengal and the deltas of the Ganges and Brahmaputra rivers).

Between 1817 and 1923 there were six cholera pandemics: 1817-23, 1826-37 (Europe and America), 1846-62, 1864-75, 1883-96, and 1900-25. In Russia cholera occurred for the first time in 1823 in Astrakhan and in 1829, in Orenburg.

During the fifth pandemic (1883-96), 800,000 people died in Russia. During the century cholera attacked about 5,500,000 persons, causing 2,322,138 deaths.

According to data furnished by WHO, 668,650 cases of cholera were registered in the countries of Asia and Africa between 1953 and 1961, 325,191 between 1959 and 1964 and 35,329 cases in 1965.

The causative agent of cholera, *Vibrio cholerae* (*Vibrio comma*), was discovered by R. Koch in 1883. Later, more than 30 varieties of the cholera vibrio and choleriform vibrios, inhabiting freshwater reservoirs, were revealed. In 1888 in Odessa N. Gamaleya isolated a vibrio (*Vibrio metschnicovi*) from the blood and gastric contents of chickens that had succumbed to a choleriform infection. The cholera vibrio is classified in the genus *Vibrio*, family *Spirillaceae*, and order *Pseudomonadales*.

Morphology. Cholera vibrios are shaped like a comma or a curved rod measuring 1-5μ in length and 0.3-0.6μ in breadth (Fig. 93). They are very actively motile, monotrichous, nonsporeforming, noncapsulated, and Gram-negative.

The cholera vibrio is subject to individual variation when it is exposed to physical and chemical factors. On artificial media and in old cultures it occurs in the form of grains, globes, rods, threads, clubs or spirals. When it is re-inoculated into fresh media, the organism assumes its initial form.

Cultivation. Cholera vibrios are aerobes. The optimum growth temperature is 37°C, and growth is arrested below 14°C and above 42°C. The organisms grow readily on alkaline media at pH 7.6-8.0,

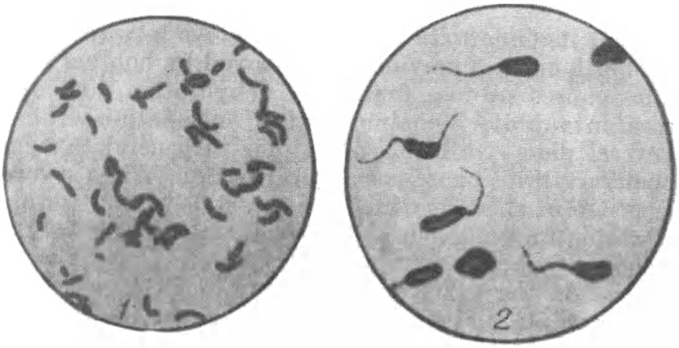


Fig. 93. *Cholera vibrio*
1—pure culture; *2*—flagellate vibrios

and on solid media the colonies are transparent with a light-blue hue, forming domes with smooth edges. On gelatin the organisms produce transparent granular colonies which, when examined under a microscope, resemble broken glass. In 48 hours the medium surrounding the colonies becomes liquefied and the colonies sink into this area. Six-hour-old cultures on alkaline meat broth and peptone water produce a pellicle, which consists of cholera vibrios.

The organism is also subjected to cultural changes. It dissociates from the S-form to the R-form, this process being accompanied by profound changes in antigenic structure.

Fermentative properties. The cholera vibrio liquefies coagulated serum and gelatin (Fig. 94), and produces indole, ammonia, and hydrogen sulphide. The organism reduces nitrates to nitrites, breaks down urea, ferments glucose, levulose, galactose, maltose, saccharose, mannitol, starch, and glycerin (late fermentation in the latter case), with acid formation, but ferments no lactose in the first 48 hours. Milk coagulation is not a constant property. True cholera vibrios do not lyse sheep and goat erythrocytes, unlike the cholera-like organisms. Haemolytic variants of choleric vibrio may occur.

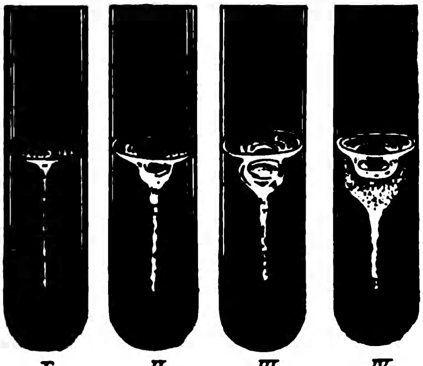


Fig. 94. *Cholera vibrio*
I-IV—subsequent stages of gelatin liquefaction

Toxin production. No exotoxin is produced by the chol-

era vibrio, but its endotoxin is highly toxic. An intravenous injection of a meat broth culture previously destroyed by holding at 55°C for 30 minutes causes profuse diarrhoea, dehydration, and opacity of the cornea in rabbits 30 minutes after inoculation. The animals die in several days exhibiting symptoms of general toxaemia.

The cholera vibrios cause haemagglutination. When several drops of a suspension of sheep erythrocytes are added to an isotonic solution containing an emulsion of 18-24-hour-old freshly isolated culture of cholera vibrios, within a few minutes the blood acquires a violet hue. At the same time the erythrocytes are agglutinated, and they flocculate and quickly settle down at the bottom of the test tube. The reaction takes place at 37 and 0°C.

Vibrio haemagglutinins are inactivated by heating at 64°C for 5 minutes. The vibrios produce enzymes which cause changes in the erythrocyte components. The effect of the organisms on the blood is very complex and varies widely depending on strain characteristics. This property is not constant, and therefore, it is not a definite criterion for differentiation between cholera vibrios and cholera-like organisms.

Antigenic structure. Cholera vibrios possess thermostable O-antigens (somatic) and thermolabile H-antigens (flagellar). There is evidence that the organisms contain 13 thermostable O-antigens. The cholera and cholera-like vibrios are differentiated by an agglutination reaction with O-agglutinating sera, which are obtained by immunization of animals with a boiled culture. Agglutination reactions performed with the usual live and motile culture which contains H-antigens may lead to diagnostic errors since the cholera vibrio H-antigens are similar to those of the cholera-like organism.

Classification. Japanese authors subdivide cholera vibrios into three types: I (Inaba), II (Hikojima), and III (Ogawa), but this classification is of no practical value nowadays.

Investigations carried out by A. Gardner and K. Venkatraman showed that on the basis of their O-antigens all vibrios can be divided into six O-subgroups: I, II, III, IV, V, and VI. The OI-subgroup comprises the true cholera vibrios and some of the El Tor vibrios (those which cause lyses of erythrocytes). All the water vibrios which produce haemolysis on blood media and some of the El Tor vibrios are included in the remaining subgroups (Table 24).

Susceptibility to lysis by the cholera bacteriophage and agglutination by the O-agglutinating serum are the most reliable properties of differentiation between the cholera and cholera-like vibrios.

Resistance. The cholera vibrio survives for a long time at low temperatures. It lives in faeces for up to a month, in oysters, crabs, on the surface of fish and in their intestines from 1 to 40 days, in water for several days, on foodstuffs from 1 to 10 days, and in the intestines of flies from 4 to 5 days.

The organism shows a low resistance to sunlight, X-rays, des-

Table 24

Differentiation of Cholera and Cholera-Like Vibrios

Type of vibrio	Fermentation within 24 hours: saccharose	mannose	starch	arabinose	Erythrocyte haemolysis	Cholera red reaction	Reaction with the cholera bacteriophage	Agglutination by the cholera C-sera
Cholera vibrios	A	A	A	—	—	+	+	+
Cholera-like vibrios	—	—	—	A	+	∓	—	—

where: A indicates carbohydrate fermentation with acid formation;
+ indicates a positive reaction;
— indicates a negative reaction;
∓ indicates that the cholera red reaction is not constant.

iccation, and high temperatures. It is destroyed instantly at 100°C, and in 5 minutes at 80°C. Cholera vibrios are highly sensitive to disinfectants, particularly to acids (e.g., a 1:10,000 solution of hydrochloric acid kills them within one minute). The organism is also very sensitive to the action of gastric juice.

Pathogenicity for animals. In nature animals are not attacked by cholera, but an intraperitoneal injection of the culture into rabbits and guinea pigs gives rise to general toxicosis and peritonitis which is followed by death.

In his experiments with rabbit-sucklings E. Metchnikoff produced a disease similar to human cholera, by oral infection. R. Koch reproduced the disease in guinea pigs previously alkalizing the gastric juice and introducing opium. An intravenous vibrio injection into rabbits and dogs gives rise to lethal toxaemia.

Pathogenesis and diseases in man. The cholera vibrios are transmitted from sick persons and carriers by food, water, flies, and contaminated hands. Via the mouth the organisms gain entrance into the small intestine, where the alkaline medium and an abundance of products of protein catabolism furnish favourable conditions for their multiplication. When the cholera vibrios perish, a large amount of endotoxin is released. This endotoxin invades the patient's blood owing to necrosis of the intestinal epithelium and the resulting condition promotes disturbance of the intestinal vegetative nerve fibres, dehydration of the body, and a development of intoxication.

Cholera is characterized by a short incubation period (from several hours and up to 6 days), and the disease symptoms include general weakness, vomiting, and a frequent loose stool. The stools

resemble rice water and contain enormous numbers of torn-off intestinal epithelial cells and cholera vibrios.

Three phases can be distinguished in the development of the disease. 1. Cholera enteritis (choleric diarrhoea) which lasts 1 or 2 days. In some cases the infectious process terminates in this period and the patient recovers. 2. Cholera gastroenteritis is the second phase of the disease. Proffuse diarrhoea and continuous vomiting lead to dehydration of the patient's body and this results in lowering of body temperature, decrease in the amount of urine excreted, drastic decrease in the number of mineral and protein substances, and the appearance of convulsions. The presence of cholera vibrios is revealed quite frequently in the vomit and particularly in the stools which have the appearance of rice water. 3. Cholera algid which is characterized by severe symptoms. The skin becomes wrinkled due to the loss of water, cyanosis appears, and the voice becomes husky and is sometimes lost completely. The body temperature falls to 35.5-34°C. As a result of blood concentration cardiac activity is drastically weakened and urination is suppressed.

In severe cases the algid period is followed by the asphyctic phase characterized by cyanosis, dyspnoea, uraemia, azotaemia, and unconsciousness (cholera coma), which lead to prostration and death. Effective treatment and proper nursing care may induce a change of the algid period to the reactive phase during which urination becomes normal, intoxication decreases, and the patient recovers. Fulminate forms of cholera (dry cholera or cholera sicca) may occur in a number of cases. These forms are characterized by the absence of diarrhoea and vomiting and result in death due to severe intoxication. Atypical and latent forms of cholera are exhibited quite frequently, particularly in children, resembling mild cases of gastroenteritis.

Nonspecific complications in cholera include pneumonia, erysipelas, phlegmons, abscesses, occasionally sepsis, etc. Among the specific complications cholera typhoid is the most menacing. It is accompanied by a rise in body temperature to 38-39°C, eruptions on the skin, vomiting and fetid loose stools. This condition causes a mortality rate of 80-90 per cent.

About 10 per cent of all cholera cases which occurred in Asian countries during the first half of 1965 were caused by El Tor vibrios. These organisms haemolyzed goat and sheep erythrocytes but in other respects were identical to true cholera vibrios.

Post-mortem examination of cholera cases reveals distinct hyperaemia of the peritoneum and serosa of the small intestine, which are covered with a sticky exudate. The mucous membrane of the small intestine is congested, peach-coloured, the intestinal epithelium is frequently desquamated, and there are haemorrhages in the submucosa. The vibrios are present in great abundance in the

intestinal wall, particularly in Lieberkühn's glands, and, not infrequently, in the gall-bladder.

The mortality rate for cholera is quite high, although with the use of antibiotics it has declined considerably. Thus, between 1919 and 1949 a total of 350,000-400,000 deaths occurred annually in all countries, while between 1950 and 1960 the corresponding figure did not exceed 10,000-15,000.

Immunity acquired after cholera is high-grade but of short duration. It is associated mainly with the presence of antibodies (lysins, agglutinins, and opsonins). The cholera vibrios rapidly undergo lysis under the influence of immune sera which contain bacteriolysins.

E. Metchnikoff attributed definite significance to phagocytosis following immunity. The normal activity of the stomach, whose contents are bactericidal to the cholera vibrio, plays an essential role in the natural defence mechanism.

Laboratory diagnosis. A strict regime is established in the laboratory. Examinations are carried out in accordance with the general rules observed for particularly hazardous diseases.

Test specimens are collected from stools, vomit, organs obtained at autopsy, water, objects contaminated by patient's stools, and, in some cases, from foodstuffs. Certain rules are observed when the material is collected and transported to the laboratory, and examination is made in the following stages.

1. Stool smears stained by a water solution of fuchsin are examined microscopically. In the smears, the cholera vibrios occur in groups similar to shoals of fish (Fig. 95).

2. A stool sample is inoculated into 1 per cent peptone water and alkaline agar. After 6 hours incubation at 37°C the cholera vibrios form a thin pellicle in the peptone water, which adheres to the glass. The pellicle smears are Gram stained, and the culture is examined for motility. A slide agglutination reaction is performed with specific agglutinating O-serum diluted in a ratio of 1 in 100. The organisms are then transferred from the peptone water onto alkaline agar for isolation of the pure culture. If the first generation of the vibrios in peptone water is not visible, a drop taken from the surface layer is re-inoculated into another tube of peptone water. In some cases with such re-inoculations, an increase in the number of vibrios is achieved.

Fig. 95. *Cholera vibrio* (stool smear)

The vibrio culture grown on solid media (alkaline agar or Dieudonné's medium) is examined for motility and agglutinable properties. Then it is subcultured on an agar slant to obtain the pure culture.

3. The organism is identified finally by its agglutination reaction with specific O-serum, determination of its fermentative properties (fermentation of mannose, saccharose, and starch), and its susceptibility to phagolysis (see Table 24).

In ascertained cholera sources rapid and auxiliary diagnostic methods are employed, in particular a rapid method for mass examination for carriers and a rapid method of laboratory diagnosis by a rise in the phage titre.

Treatment. Cholera patients are given sulphonamides (sulphadiazine, sulphathiazole, sulphaxuxidine, phthalylsulphacetimide, sulgine, etc.), antibiotics (chloromycetin, levomycetin, chlortetracycline, oxytetracycline, streptomycin, etc.), and choleric bacteriophage active against the given type or strain. Pathogenic therapy is of great importance, and includes control of dehydration, hypoproteinaemia, disturbances of metabolism, conditions due to toxicosis (in particular acidosis) by injections of physiological and saline solutions, plasma or dry serum, and glucose, recommendation of warm baths, and prescription of cardiotonic and vasomotor drugs.

Prophylaxis. The following measures are carried out in cholera foci.

1. Recognition of the first cholera cases, accurate registration of all cases and information of higher Public Health authorities.
2. Isolation and hospitalization of patients and carriers in accordance with special regulations, observation of contacts and their compulsory treatment with bacteriophage.
3. Routine and terminal disinfection of departments for cholera patients and of disease foci.
4. Protection of water-supply sources, enforcement of sanitary inspection of catering establishments, and fly control.
5. Strict observance of personal hygiene; boiling or chlorination of water, disinfection of dishes, and washing of hands.
6. Specific prophylaxis: vaccination with monovalent cholera vaccine or polyvaccine; bacteriophage treatment of cholera patients, contacts, and medical personnel who work in the departments of infectious diseases or help with epidemic control measures.

As a result of active prophylactic measures being conducted, there has been no incidence of cholera in the USSR since 1926.

PATHOGENIC SPIRILLA

Spirillum minus (V. Carter, 1888), the causative agent of sodoku (rat-bite fever), is the only representative of the pathogenic spirillae. It belongs to the family *Spirillaceae*, and genus *Spirillum* (Fig. 96*a*). Some investigators classify the organism in the order *Spirochaetes*.

Morphology. *S. minus* is a short, thick, rigid and motile organism 2-3μ in length and 0.3-0.5μ in breadth which possesses a tuft of flagella at each end (Fig. 96*b*). They are Gram-negative and are stained pink-violet by the Romanowski-Giemsa stain.

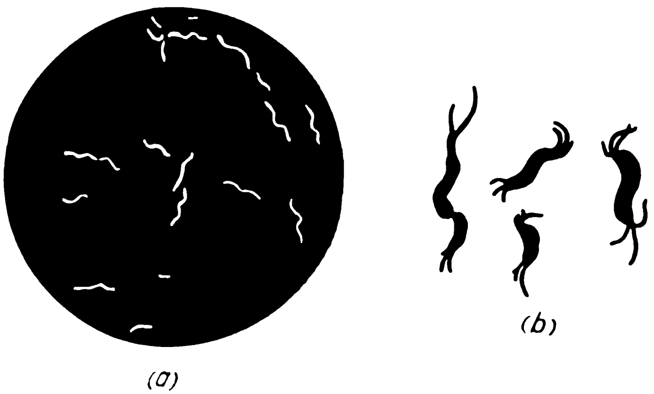


Fig. 96. Spirilla in dark-field illumination (*a*) and with flagella at their ends (*b*)

Cultivation. The pathogenic spirillae have not so far been maintained in culture, displaying only very poor growth in haemolysed rabbit blood.

Fermentative properties, toxin production, antigenic structure, and classification have not yet been studied. Spirillae produce no

soluble toxin. An endotoxin is thought to be responsible for all pathological changes.

Resistance. Being extremely parasitic, the causative agent of sodoku is unstable in the bodies of warm-blooded animals. It is preserved with great difficulties for several days in a human blood clot at 37°C.

Pathogenicity for animals. *S. minus* is pathogenic for white mice, rats, and guinea pigs. The organisms are found in sick mice, cats, and dogs.

Pathogenesis and diseases in man. The causative agent localizes in the wound and gives rise to an inflamed area at the site of penetration. It affects the lymph nodes and may gain entrance into the internal organs (kidneys, adrenal glands, liver, ovaries, and cerebral membranes) in the blood.

The disease is characterized by its sudden onset, pyrexia (39-41°C), pain at the site of the bite, and sometimes infiltrations, and ulcerations. The pyrexial period lasts 3 to 5 days and is followed by apyrexia; in 2 or 3 days the temperature rises again. The number of attacks varies from 4 to 10. The second or third attack is followed by a polymorphous skin rash, miositis, arthritis, and inflammation of the mouth and eye mucosa.

Immunity has not been studied sufficiently. The sera of patients and convalescents have been found to contain lysins and complement-fixing antibodies.

Laboratory diagnosis comprises: (a) microscopic examination by dark-field illumination of exudate obtained from the area of inflammation (i.e., the area of the bite); (b) inoculation of experimental animals (guinea pigs and white mice) with the contents of the affected lymph nodes. Spirillae appear in the blood and abdominal cavity exudate of sick animals.

Treatment. Patients are prescribed penicillin and arsenic preparations (novarsenol). Good effects are obtained with streptomycin, chlortetracycline, and oxytetracycline.

Prophylaxis depends upon general measures—rat killing and personal hygiene.

CAPSULATED BACTERIA

The family *Enterobacteriaceae*, genus *Klebsiella* include bacteria which are capable of producing capsules when present in the host's body or on nutrient media.

Morphology. The capsulated bacteria are thick short bacilli 2-5μ in length and 0.3-1.25μ in breadth. They have rounded ends, are nonmotile and devoid of spores. They occur mainly in pairs but may be seen frequently as single organisms, and are normally surrounded by a capsule. They stain readily with all aniline dyes and are Gram-negative.

Cultivation. The capsulated microbes are facultative aerobes, which grow readily on common nutrient media at pH 7.2 and at a temperature of 35-37°C. No growth is shown below 12°C or above 37°C. The organisms are capable of synthesizing all amino acids essential for their growth. They form turbid mucilaginous colonies on agar and produce intense turbidity in broth. After 2 or 4 hours the capsulated bacteria show a characteristic arrangement in the young colonies (Fig. 97). The young colonies are studied with a dry lens (lens No. 7) in pieces of agar taken from Petri dishes. The

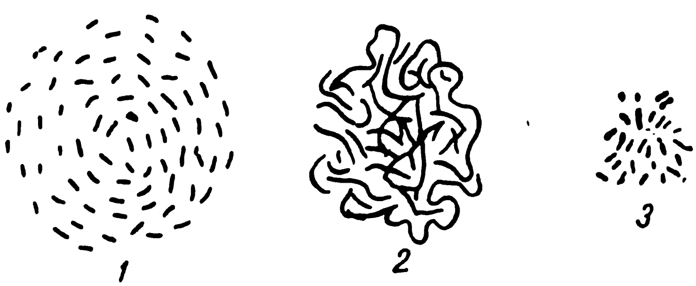


Fig. 97. Young colonies of capsulated bacteria

1—causative agent of rhinoscleroma; *2*—causative agent of pneumonia; *3*—nonfermenting bacterium

agar-microscopy method is used for differentiation of capsulated bacteria.

The capsulated bacteria may lose their capsules by prolonged subculture on 50 per cent bile broth, and acquire them again by passage through white mice. The organisms dissociate into S- and R-forms when they are exposed to the action of low temperatures, bacteriophage, chemical substances, bile, and antiserum or when they are frequently subcultured.

Fermentative properties. The capsulated bacteria do not liquefy gelatin and produce no indole or hydrogen sulphide. They reduce nitrates to nitrites and decompose urea. Milk is not always curdled. The organisms ferment carbohydrates, producing both acid and gas or, sometimes, only acid. Glucose and mannitol fermentation is usually a constant property.

Toxin production. Capsulated bacteria produce no soluble toxins, their toxicity being associated with an endotoxin.

Antigenic structure. Capsulated bacteria contain three types of antigens: capsular (K-antigen), smooth somatic (O-antigen), and rough somatic (R-antigen). The K- and O-antigens are carbohydrates, and the R-antigen is a protein. The O-antigen is subdivided into three groups: O-group 1, O-group 2, and O-group 3. The O-group 1 and the coli bacilli possess common antigens.

The K-antigen consists of 14 antigens (1, 2, 3, 4, etc.). It can be extracted from 4- to 6-hour-old broth cultures previously killed with a 0.5 per cent formalin solution, alcohol, or acetone. The organisms are differentiated by the presence of O- and K-antigens. An agglutination reaction with the noncapsulated strain which contains O-antigens and the complement-fixation reaction with the capsular antigen are performed for antibody detection.

Classification of capsulated bacteria is presented in Table 25.

Resistance. Capsulated bacteria survive at room temperature for weeks and even months. When heated to a temperature of 65°C they are destroyed in one hour. The organisms are susceptible to treatment with solutions of chloramine, phenol, citral, and other disinfectants.

Pathogenicity for animals. Among the experimental animals white mice are most susceptible. They die in 24-48 hours following inoculation, displaying symptoms of septicaemia. Severe inflammation and enlargement of the spleen and liver are found at autopsy. Capsulated bacteria are found in abundance in smears made from organs and blood. The pathogenicity of capsulated bacteria is associated with the capsule, and bacteria which have lost their ability to produce capsules become nonpathogenic and are rapidly exposed to the action of phage when injected into the animal body.

Pathogenesis and diseases in man. Three species of capsulated bacteria play a most important role in human pathology: the causative agents of pneumonia, ozaena, and rhinoscleroma.

Table 25

Differentiation of Capsulated Bacteria

Bacteria	Microscopical structure of colonies on agar	Growth in bile or in 50% bile broth	Fermentation of carbohydrates				
			lactose	glucose	saccha-rose	mannitol	maltose
Klebsiella pneumoniae (K. Friendländer, 1888)	Form loops	+	AG	AG	AG	AG	AG
Klebsiella ozaenae (R. Abel, 1893)	Concentrically scattered	+	A	A	A	A	A
Klebsiella rhinoscleromatis (S. Frisch, N. Volkovitch, 1882)	Concentrical	—	—	—	A	A	A
Aerobacter aerogenes (M. Beijerinck, 1900)	Terraced	+	AG	AG	AG±	AG	AG

where: A indicates acid;
AG indicates acid and gas;
\+ indicates growth in bile;
— indicates absence of fermentation and growth.

PNEUMONIA BACTERIA

Klebsiella pneumoniae—the morphological characteristics are given on p. 375. The organisms grow readily on solid media, producing opaque mucilaginous colonies. In young colonies grown on agar they occur in loops (Fig. 98) and are serologically heterogeneous.

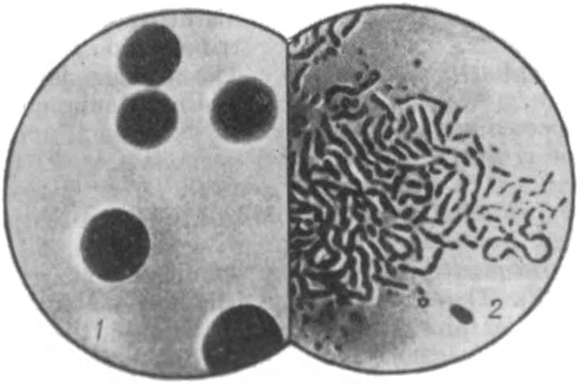


Fig. 98. *Klebsiella pneumoniae*, colonies (*1*) and loop-like arrangement in 3-hour-old culture (*2*)

Infected guinea pigs and white mice exhibit septicaemia. The causative agents are found in the blood and tissues, types A and B being most virulent.

K. pneumoniae is responsible for pneumonia. Pneumonia (bronchopneumonia) involves one or several lung lobes, sometimes producing fused foci and lung abscesses. The death rate is quite high. In some cases the organisms may be responsible for meningitis, appendicitis, pyaemia, mastoiditis, and cystitis. They may also cause inflammation in cases of mixed infections.

OZAENA BACTERIA

Klebsiella ozaenae—the morphological characteristics are given on p. 375 (Fig. 99). In young colonies the organisms are concentrically scattered. It is assumed that they are responsible for rhinitis which is characterized by an offensive nasal discharge. *K. ozaena* affects the mucous membranes of the nose, nasal sinuses, and conchae. This results in production of a viscid discharge which dries up and forms thick scabs with an offensive odour. These scabs make breathing difficult.

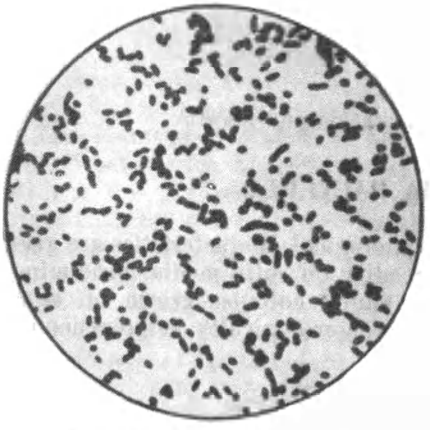


Fig. 99. *Klebsiella ozaenae*

Ozaena is a mildly contagious disease and is transmitted by the air-droplet route. It is possible that other factors (trophical and endocrine disturbances, etc.) also contribute to its development. The disease is prevalent in Spain, India, China, and Japan and occurs sporadically in the USSR.

RHINOSCLEROMA BACTERIA

Klebsiella rhinoscleromatis—the morphological characteristics are given on p. 375. The organisms are differentiated by their growth on agar and other properties. In young colonies they are arranged concentrically (Fig. 100).

The rhinoscleroma bacteria occur in tissue nodes (infectious granulomas) in the form of short capsulated microbes. They are localized intra- and extracellularly.

The organisms are responsible for chronic granulomatosis of the skin and mucous membranes of the nose, pharynx, larynx, trachea, and bronchi, with the formation of granulomas. Rhinoscleroma is a mildly contagious disease. It prevails in Austria and Poland and occurs sometimes in Belorussia, the Ukraine, Siberia, and Central Asia. Treatment is a matter of great difficulty and envolves complex therapeutic measures which must be carried out over a long period of time.

Immunity. Diseases caused by capsulated pathogenic bacteria leave low-grade immunity. Agglutinins and complement fixing

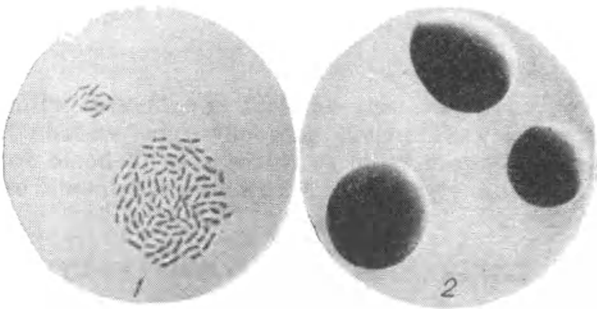


Fig. 100. *Klebsiella rhinoscleromatis*

1—agar microscopy of 3-hour-old culture; *2*—colonies of 18-hour-old culture

antibodies are present in the blood of ozaena and rhinoscleroma patients, but their defence role is negligible. The absence of an infectious immunity is probably the reason for the chronic nature of these diseases.

Laboratory diagnosis includes the following methods.

1. Microscopic examination of smears made from sputum (from patients with pneumonia), nasal mucus discharge (from patients with ozaena), and tissue specimens (from patients with rhinoscleroma). Pathohystological examination of infiltrates reveals a great number of peculiar giant Mikulicz's cells which contain capsulated bacteria in a gelatin-like substance. The material is collected with a loop or cotton-wool swab, having previously scarified the mucosa surface.

2. Isolation of the pure culture and its identification by cultural, biochemical, phagocytolytic, and serological properties.

3. Complement-fixation reaction with patients' sera and capsular antigen. This reaction yields positive results most frequently. Sera diluted in ratios from 1:5 to 1:400 are used for the agglutination reaction with a noncapsulated strain.

4. The allergic skin test is employed as an additional test, but is less specific than the agglutination reaction or the complement-fixation reaction.

Treatment. Patients are treated with streptomycin, levomycetin, colimycin, tetracycline, and antimony preparations (solusurmin). Vaccine therapy is also employed. The vaccine is prepared from capsulated bacteria strains which have been killed by heat treatment.

Prophylaxis is ensured by recognition of the early stages of ozaena and rhinoscleroma, active antibiotic therapy, and prevention of healthy individuals from being infected by the sick.

CAUSATIVE AGENTS OF INFLUENZA, SOFT CHANCRE, AND WHOOPING COUGH

The family *Brucellaceae* includes the causative agents of influenza, soft chancre, and whooping cough. They grow only in the presence of blood (haemoglobin) or other growth factors. If these factors are absent the organisms cannot live and multiply.

CAUSATIVE AGENT OF INFLUENZA

M. Afanasyev in 1889 and R. Pfeiffer and S. Kitasato in 1892 encountered very small Gram-negative bacilli in the sputum of patients during an influenza pandemic. For forty years these organisms were mistakenly considered to be responsible for influenza. Later they were shown to be concomitants of influenzal infections and the causative agents of acute catarrhs and secondary infections.

Morphology. The influenza bacilli (*Haemophilus influenzae*) are very small organisms, measuring 0.5-2μ in length and 0.2-0.3μ in breadth. They appear as small rods with rounded ends. The organisms are nonmotile, nonsporeforming, and Gram-negative. The virulent smooth strains are capsulated. The bacilli stain relatively well with dilute fuchsin, the ends staining more intensely than the central portion.

H. influenzae are pleomorphous, and sometimes grow in the form of long threads with round- or spindle-shaped swellings.

Cultivation. The organisms are aerobes and facultative aerobes. They do not grow on common nutrient media but multiply readily on blood agar at pH 7.3-7.5 and at a temperature of 37°C. The extremes of temperature for growth are 25° and 43°C. Small transparent colonies resembling drops of dew become visible on the medium after 24 hours (Fig. 101). White flakes and slight turbidity are produced in blood broth.

H. influenzae may be cultivated on common media together with staphylococci, *B. coli*, *B. prodigiosum*, and *Vibrio metschnikovii*,

which secrete vitamins (growth factors) into the medium, and for this reason are known as microbe-feeders.

On chocolate agar (heated blood agar) *H. influenzae* produces large transparent flat colonies. According to the form of their colonies, two types of bacilli are distinguished: the smooth bacilli (typical) and the rough bacilli (atypical). *H. influenzae* grows on nutrient media only in the presence of two factors, the so-called X-factor which is thermostable and survives heating up to 120°C and the V-factor, which is thermolabile and occurs in blood, fresh potatoes, animal and vegetable tissues, and in a large number of bacteria.

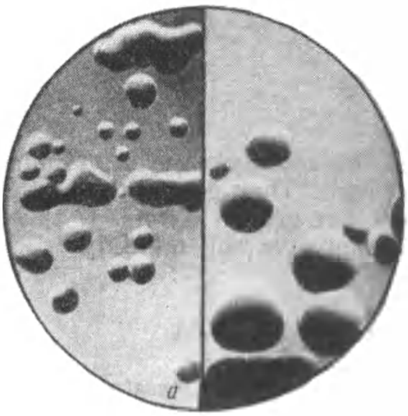


Fig. 101. Colonies of *Bord. pertussis* (*a*) and *H. influenzae* (*b*)

Atypical forms often appear in cultures. M- and N-strains can be distinguished. The M-strains are more virulent and are isolated more frequently from meningitis patients, whereas the N-strains are less virulent and are usually found in the nasal mucus.

Fermentative properties. *H. influenzae* reduces nitrates to nitrites. The smooth typical strains produce indole and sometimes cause slow glucose fermentation, with acid formation.

Toxin production. The bacilli produce no exotoxin. Their pathogenicity is associated with an endotoxin which is liberated as a result of bacterial disintegration.

Antigenic structure and classification. The organisms are serologically heterogeneous. The smooth forms are characterized by type specificity due to the presence of polysaccharides. On the basis of their antigenic structure, the bacilli are differentiated into 6 (a, b, c, d, e, f) serological types which are detected by the precipitin reaction between immune sera and capsular material. The rough atypical strains are heterogeneous, and their antigenic structure has not been sufficiently studied.

Resistance. *H. influenzae* are not very resistant organisms, and can survive only for a short period outside the body. The organisms are susceptible to physical and chemical factors and are easily killed by exposure to a temperature of 59°C, sun rays, desiccation, and disinfectants.

Pathogenicity for animals. Experimental animals (white mice) infected with *H. influenzae* cultures display symptoms of toxaemia. The bacteria do not normally invade the blood.

Pathogenesis and diseases in man. *H. influenzae* give rise to acute catarrhs of the upper respiratory tract in combined action with other bacteria (pneumococci, staphylococci, streptococci, adenoviruses, etc.). Decrease in temperature facilitates the development of the infections, and for this reason they are known as colds and seasonal infections, and prevail during the cold months.

A sudden drop in temperature and exposure to the effect of influenza viruses weaken the general immunobiological defence mechanisms of the body, as a result of which certain bacteria which are present as commensals in the human throat become more active.

In the human body *H. influenzae* localize in the mucous membranes of the respiratory tract and bronchi. They occur extra- and intracellularly and are sometimes found in the blood. The organisms are isolated quite frequently from acute catarrhal cases and are at times responsible for acute inflammatory conditions (laryngitis, tonsillitis, bronchitis, pneumonia, otitis, meningitis, etc.). They also give rise to various postinfectional complications, particularly in children.

Immunity. Immunity acquired after *H. influenzae* infections has not been sufficiently studied. It is thought that acute catarrhal conditions produce no immunity. This is accounted for by the multibacterial aetiology of the disease. The commensals present in the upper respiratory tract and nose may cause various lesions in the weakened organism known under the common name of catarrhs.

Insusceptibility to acute catarrhs of the respiratory tract depends on the condition of the body's physiological defence mechanisms as well as on the ability of the body to endure changes in the temperature, humidity, and other factors of the environment.

Laboratory diagnosis. Specimens from sputum and nasal discharge serve as test material. Mucus from the tonsillar and nasopharyngeal mucosa is collected with a cotton-wool swab, and the following procedures are carried out:

(1) smears are prepared from sputum and stained with fuchsin for 5-10 minutes;

(2) purulent sputum clots washed in 0.85 per cent saline solution are inoculated into blood agar, chocolate agar, or Levinthal's medium. The material may be plated by the cough-plate method when an open plate of medium is held at a distance of 5-8 cm in front of the patient's mouth when he coughs. The cultures are incubated at 37°C. From 15 to 25 units of penicillin are added to the medium to inhibit the growth of coccal microflora. The isolated culture is differentiated from whooping cough bacilli by its biochemical and antigenic properties. *Haemophilus parainfluenzae* is present as a commensal in the respiratory tract mucosa of humans and cats. This organism is usually nonpathogenic.

Treatment. Patients are given streptomycin together with sulphonamides, and also chloromycetin, chlortetracycline, oxytetra-

cycline, polymyxin, neomycin, and sulphonamides. Disinfectant gargles are also prescribed.

Prophylaxis includes prevention of cooling and body hardening by systematic physical exercises. Physical culture and sports, sufficient nourishment, with a full-value vitamin content in particular, and observance of rules of hygiene at work and in everyday life play an important part in the prophylaxis of catarrhs.

Conjunctivitis, caused by *Haemophilus aegyptius*, occurs in summer mainly in countries with a warm climate.

CAUSATIVE AGENT OF SOFT CHANCRE

The soft chancre bacillus (*Haemophilus ducreyi*) was discovered by P. Ferrari in 1885. Its aetiological role was shown in experiments in 1887 by O. Petersen, and described in detail by A. Ducrey in 1889, and studied by P. Unna in 1892.

Morphology. The organism is oval in shape and measures 1.5-2μ in length and 0.5μ in breadth. In smears from ulcers it occurs in groups or long chains (Fig. 102). The organism forms neither spores, capsules, nor flagella. It is Gram-negative and exhibits bipolar staining.

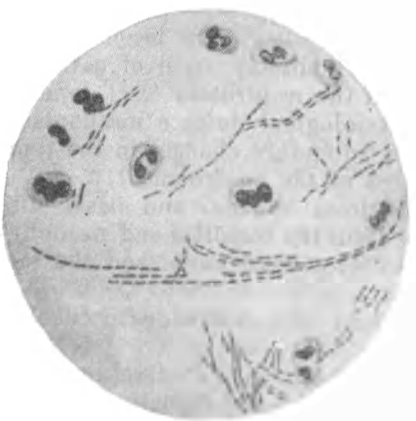


Fig. 102. Causative agent of soft chancre (smear from ulcer discharge)

Cultivation. The causative agent of soft chancre is an aerobe and a facultative aerobe. It does not grow on common media but grows on blood agar at 37°C (35°-38°) and pH 7.2-7.8, on Martin's broth medium containing 20 per cent defibrinated blood, and on medium consisting of one part of 5 per cent glycerin agar and four parts of fluid egg medium. On blood agar the organisms are haemolytic and produce small, round, globe-shaped isolated colonies which measure 1-2 mm in diameter.

Fermentative properties. The organism is nonproteolytic. It ferments glucose, lactose, saccharose, and mannitol, with acid formation.

Toxin production. No soluble toxin is produced, all pathological changes being caused by an endotoxin.

Antigenic structure and classification have not been elaborated. The soft chancre bacillus must be differentiated from the haemophilic organisms found in cases of ulcerative lesions. The Miyahara

bacterium does not form chains, and the causative organism of Frei's disease is more fragile, monomorphous, and does not occur in parallel arrangement.

Resistance. The soft chancre bacillus is sensitive to various environmental factors. It withstands 55°C for 15 minutes and is destroyed in dilute disinfectant solutions.

Pathogenicity for animals. Monkeys are the only animals susceptible to *H. ducreyi*, and display a mild form of the disease. Guinea pigs and rabbits are insusceptible to inoculation.

Pathogenesis and disease in man. Soft chancre is a typical venereal disease and is transmitted via the genital organs. Individuals with an acute or chronic form of the disease are sources of infection.

The organism multiplies in the skin or mucous membranes of the genitalia. An inflammatory process develops at the site of penetration and is followed by ulceration. The ulcer is soft, with uneven edges and a purulent discharge, and is painful. Invasion of the adjacent parts of the body by the bacillus results in formation of a great number of painful ulcers and lesions of the lymphatic vessels with the development of lymphangitis and lymphadenitis. In the absence of ulcers the organism may localize in the mucous membranes of the vagina, cervix uteri, and the urethra.

Immunity. The disease leaves no immunity, although it gives rise to the production of complement-fixing antibodies and development of allergy.

Laboratory diagnosis comprises the following:

(1) microscopical examination of excretions obtained from deep ulcer layers, the smears being stained with methylene blue or with the Gram stain. In the microscope long chains of Gram-negative bacilli can easily be seen;

(2) inoculation into blood agar, isolation of the pure culture and its identification by the agglutination reaction with specific serum from the patients;

(3) employment of the allergic reaction (intracutaneous test) with an antigen derived from the soft chancre bacilli; a papule surrounded by a zone of inflammation will appear at the site of injection of the antigen in 24-48 hours after inoculation.

Treatment. Sulphonamides and antibiotics (penicillin, streptomycin, chlortetracycline, oxytetracycline, and chloromycetin) are prescribed.

Prophylaxis is ensured by social changes which have eliminated poverty, unemployment, and prostitution in the USSR and socialist countries, improved cultural and hygienic standards of the population, established sound family relations, and bettered conditions of life.

In the USSR this disease has been eliminated since 1951.

CAUSATIVE AGENT OF WHOOPING COUGH

The causative agent of whooping cough (*Bordetella pertussis*) was discovered and isolated in pure culture from patients by J. Bordet and O. Gengou in 1906.

Morphology. The organisms are small oval-shaped nonmotile rods, 0.2-0.3μ in breadth and 1μ in length. They are nonsporeforming and produce no capsules. The bacillus stains poorly with the usual aniline dyes, the ends staining more intensively. The organism is Gram-negative and less pleomorphous than *H. influenzae*.

Cultivation. *Bord. pertussis* shows no growth on ordinary media but can be cultivated readily on glycerin-potato or blood agar media under aerobic conditions at pH 6.8-7.4 and at a temperature of 35-37°C. The organism does not grow at temperatures below 20° and above 38°C. The colonies are small, convex, and glistening, resembling globules of mercury (Fig. 103), and may be granular or smooth. In blood broth the organisms produce turbidity and a small precipitate. At present, casein-charcoal medium is considered a very useful medium on which *Bord. pertussis* grows quite readily without the necessity of adding blood.

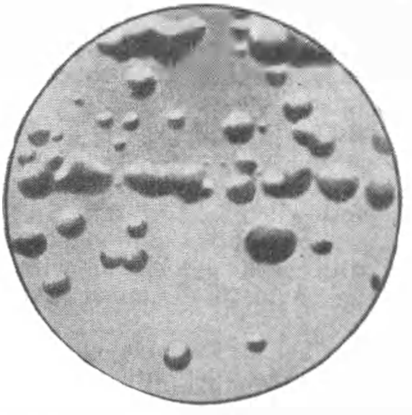


Fig. 103. Colonies of causative agent of whooping cough

Bord. pertussis, grown on media which do not contain blood, dissociate into four different phases: the first and second phases are virulent cultures, while the third and fourth are avirulent.

Colonies of the first and second phases (S-forms) are small (1-2 mm), convex, and have smooth borders. Microscopic examination of smears reveals the presence of small ovoid-shaped organisms with rounded ends. They are readily agglutinated by homologous sera to the titre. Colonies of the third and fourth phases (R-forms) are large (3-4 mm), flat, and glistening. The organisms from these colonies are not agglutinated by sera of the first and second phases but are agglutinated by homologous sera to the titre.

Fermentative properties. The bacteria do not ferment proteins, carbohydrates, or urea, but produce catalase.

Toxin production. The organisms produce a thermostable toxin, and some strains from the smooth colonies also produce a thermolabile toxin.

Bord. pertussis coagulates human, calf, sheep, and rabbit blood plasma.

Antigenic structure. *Bord. pertussis* contains a somatic O-antigen and surface capsular antigens (a, e, f, and h). The antigenic properties of the bacteria from smooth colonies differ distinctly from those of the rough colony organisms. Strains from smooth colonies of the first phase possess the most valuable immunogenic properties. Bacteria of the smooth colonies contain a thermolabile relatively stable capsular antigen and a thermostable capsular antigen. The second and third phases are intermediate and the fourth phase of dissociation is related to the rough form which has lost its virulence.

Classification. Apart from the typical bacilli of whooping cough, other species occur (*Bordetella parapertussis*, etc.) which also produce the disease in children (see Table 26).

Resistance. *Bord. pertussis* is very sensitive to environmental factors. It withstands exposure to direct sunlight for one hour and a temperature of 56°C for 10 to 15 minutes. It is relatively rapidly destroyed in 3 per cent solutions of phenol and lysol.

Pathogenicity for animals. Animals are insusceptible to *Bord. pertussis* in nature. Whooping cough has been reproduced experimentally in monkeys and young dogs, the culture being isolated from the bronchi. The disease caused fever and catarrh. Laboratory animals (rabbits, guinea pigs, and white mice) infected with the cultures exhibit toxaemia and haemorrhagic foci in the internal organs.

Pathogenesis and diseases in man. Whooping cough is transmitted from the patient to a healthy individual by the air-droplet route. Patients are most contagious in the catarrhal stage. Various objects in the vicinity of the patient are insignificant in relation to the transmission of the infection as *Bord. pertussis* cannot withstand external environmental factors. Patients with atypical clinical forms of the disease and healthy individuals who have become temporary carriers of the organisms as a result of contact with patients are also sources of infection.

Whooping cough is a severe infectious disease of childhood. It is characterized by typical symptoms and a cyclic course (three stages):

(a) catarrhal stage, lasting about 2 weeks;

(b) paroxysmal (convulsive) stage which is accompanied by a paroxysmal cough and lasts for another 4 or 6 weeks;

(c) final or convalescent stage, lasting for 2 or 3 weeks.

The organisms enter the body through the upper respiratory tract and multiply in its mucosa. The blood is not invaded. The organisms liberate toxins which cause inflammation of tracheal and bronchial mucosa. The toxins stimulate the receptors in the mucous membranes and give rise to a continuous flow of impulses

to the central nervous system thus forming a stable focus of excitation. According to A. Ukhtomsky, the focus is dominant in character. It attracts stimulations from other parts of the nervous system, and, as a result, paroxysmal cough is produced not only by the effect of specific (toxins of pertussis bacilli) stimulations but also by nonspecific stimulations (sound, injection, examination, etc.). The dominant focus dies out when stronger excitation foci appear. The attacks disappear when the patient changes his surroundings or residence, when he is taken on an air trip, or when he is occupied in a game.

Immunity. The disease leaves a stable immunity of long duration, agglutinins, precipitins, and complement-fixing antibodies accumulating in the blood.

Laboratory diagnosis. Patient's sputum and discharge from the nasopharyngeal mucosa are examined. Specimens are collected with special swabs. Sputum is inoculated into Bordet-Gengou medium, milk-blood agar, casein-hydrolysate medium, casein-charcoal medium, etc., and antibiotics (penicillin, etc.) are added to inhibit the growth of other microflora. Favourable results are obtained by the cough-plate method. After 2-5-days' incubation on Bordet-Gengou medium the organism produces typical small colonies which are convex, glistening, and resemble mother-of-pearl or globules of mercury.

The isolated pure culture is identified by its morphological, cultural, biochemical, antigenic, and biological properties (Table 26).

The causative agent of whooping cough does not ferment carbohydrates. Intracutaneous infection of albino rabbits results in the appearance of haemorrhages at the site of injection. Intraperitoneal infection is fatal to guinea pigs. When specimens obtained from patients during the catarrhal stage or in the first week of the paroxysmal stage are inoculated onto media, growth of pertussis bacilli is obtained in 75-100 per cent of cases.

Agglutination and complement-fixation reactions are employed beginning from the second week of the disease. These reactions are used for identifying both typical and atypical cases of the disease. The allergic test is also performed in which 0.1 ml of the antigen is intracutaneously injected into the patient, and an erythematous reaction measuring 2 cm in diameter and an infiltrate develop at the site of injection in 16-20 hours.

Treatment. Patients are treated with antibiotics (streptomycin, synthomycin, levomycetin, oxytetracycline, chlortetracycline, and tetracycline), human serum, gamma-globulin, and vitamins. Children undergoing treatment should have sufficient fresh air, and for this purpose the room must be frequently ventilated and the child taken for walks.

Prophylaxis is ensured by early recognition and isolation of children with whooping cough. Chemical disinfectants are not used

Table 26

Differentiation of *Bord. pertussis, Bord. parapertussis* and *H. influenzae*

	Bordetella pertussis	*Bordetella parapertussis*	*Haemophilus influenzae*
Reduction of nitrates to nitrites	No reduction	No reduction	Reduction
Changes in litmus milk	Alkaline reaction on the 12-14th day	Alkaline reaction on the 2-4th day	
Utilization of citrates as carbon source	—	+	+
Fermentation of urea	No fermentation	Normally fermentation	
Fermentation of carbohydrates	No fermentation	No fermentation	Ferments glucose, with acid formation
Production of catalase	Production	Production	No production
Pathogenicity for rabbits (on intracutaneous infection)	Causes necrosis	Causes necrosis	Causes an infiltrate or erythema, necrosis is seldom caused

due to the low resistance of the causative agent. The patient's room should be regularly ventilated. General measures are quite frequently of little effect since whooping cough is a very contagious disease.

Specific prophylaxis is performed by vaccinating children with a pertussis-diphtherial vaccine. One millilitre of the vaccine contains 40,000 millions of killed pertussis bacterial cells and 60 units of purified diphtherial toxoid. A monovalent pertussis vaccine is also used. This vaccine is a suspension of pertussis bacilli (20,000 million per 1 ml), and is prepared from bacteria grown on Bordet-Gengou medium or on semisynthetic media and killed with merthiolate or formalin. The vaccine is injected subcutaneously three times at intervals of 30 days (1 ml per injection).

At present a compound vaccine against whooping cough, diphtheria, and tetanus is employed.

CAUSATIVE AGENT OF ANTHRAX

In Russia the disease was known as Siberian sore owing to a large epidemic between 1786 and 1788 in the Urals, which has been described by S. Andreyevsky. In 1875 the disease caused the death of 100,000 horses in Siberia. In Germany the infection is known as spleen fever.

The agent responsible for anthrax (*B. anthracis*) was described by A. Pollander (Germany) in 1849, by K. Davaine (France) in 1850, and by F. Brauell (Russia) in 1854. Studies of anthrax were originated by R. Koch (1876), L. Pasteur (1881), and L. Tsenkovsky (1883). *B. anthracis* belongs to the family *Bacillaceae* and order *Eubacteriales*.

Morphology. Anthrax bacilli are large organisms, measuring 3-10 μ in length and 1-1.3 μ in breadth. In the body of animals and man they occur in pairs or in short chains, while in nutrient media they form long chains (Fig. 104*a*). In stained preparations the ends of the bacilli appear either to be sharply cut across or slightly concave, resembling bamboo canes with elbow-shaped articulations.

Fig. 104. Anthrax bacilli

a—smear from sporogenous cultures; *b*—structure of the colony border; *c*—smear from post-mortem specimen

The bacilli are nonmotile. Outside the host's body they produce oval-shaped central spores which are smaller in diameter than the bacillus. Spores are best produced in the presence of oxygen at 30-40°C. No spores are produced in the body of man or animal or at temperatures above 43 and below 15°C.

It has been ascertained that spores may germinate into vegetative forms during the warm months under favourable conditions, and transform again into spores in the autumn.

In the bodies of man and animals the bacilli produce capsules which surround a single organism or are continuous over the whole chain (Fig. 104*c*; see Fig. 117, *9*). Capsules are also produced on nutrient media which contain blood, serum, egg yolk, or brain tissue. The capsule which contains specific proteins provides a defence mechanism and determines the virulence of the organism. The anthrax bacillus readily stains with all aniline dyes and is Gram-positive.

Cultivation. The bacillus is aerobic and facultatively aerobic. The optimum growth temperature is 37°C, and the organism does not grow below 35° and above 43°C. It grows well on all ordinary media at pH 7.2-7.6. On meat-peptone agar the bacilli form rough colonies (R) which have uneven edges and resemble the head of a medusa (Fig. 104*b*). The edges of the colonies have the appearance of locks of hair or a lion's mane. The smooth S-forms possess low virulence or are completely avirulent and noncapsulated in the body of the host.

Broth cultures of the anthrax bacillus produce flocculent growth resembling cotton wool which sinks to the bottom of the tube or flask, leaving the broth clear. Spore production is inhibited by adding a 1 per cent calcium chloride solution to the medium, and is stimulated by the presence of neutral sodium oxalate.

The anthrax bacillus undergoes a morphological change when it transforms from the R-form to the S-form. It loses its ability to form chains in smears and occurs in coccal and diplobacillary forms or the cells are arranged in groups. Incubation at 42.5°C produces thread-like, nonsporeforming forms of low virulence. On meat-peptone agar containing penicillin the bacilli break up into globules which are arranged in the form of a necklace (a pearl necklace). The anthrax bacilli transform usually from the R-form (typical, with rough colonies, and virulent) to the S-form (atypical, with smooth even-edged colonies, and avirulent) through the intermediate O-form (with mucilaginous, pigmented, and patterned colonies).

Fermentative properties. The anthrax bacilli possess great biochemical activity. They contain the enzymes—dehydrogenase, lipase, diastase, peroxidase, and catalase. In gelatin stab-cultures growth resembles an inverted fir tree (Fig. 105), the gelatin being liquefied in layers. The organisms cause late liquefaction of coagulated serum and produce ammonia and hydrogen sulphide. They slowly reduce nitrates

to nitrites and coagulate and peptonize milk. The organisms ferment glucose, levulose, saccharose, maltose, trehalose, and dextrin, with acid production.

Toxin production. *B. anthracis* does not produce a soluble toxin. The capsular matter is very toxic, containing Bail's aggressins, and loss of the capsule leads to loss of virulence.

Some *B. anthracis* strains have been shown to produce a toxin (lethal factor) in the bodies of animals. Intraperitoneal or intravenous injections of small doses of sera obtained from guinea pigs which had died from anthrax are fatal for white mice and guinea pigs.

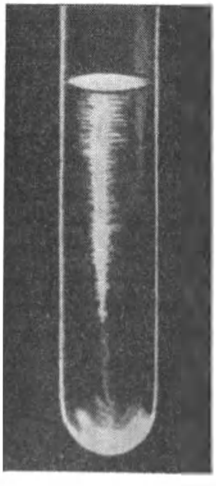


Fig. 105. Growth of *Bac. anthracis* on gelatin stab

Antigenic structure. The anthrax bacillus contains a protein (P) and a polysaccharide (C) antigen. The polysaccharide antigen is present in the bacterial cell, while the protein antigen is in the capsule.

The polysaccharide antigen consists of α-glucosamine, galactose, and acetic acid and is thermoresistant. It survives for long periods in tissues obtained from corpses. Ascoli's thermoprecipitin test is based on this principle. A boiled *B. anthracis* extract contains a polysaccharide fraction (thermoresistant) which yields a precipitin reaction with the precipitant serum.

The capsule contains a protein-like substance, a polypeptide, which contains α-glutamic acid.

In animal bodies and on media containing tissue extracts and plasma, *B. anthracis* produces a peculiar antigen (a protective antigen). This antigen is a nontoxic thermolabile protein which possesses marked immunogenic properties. It gives rise to the formation of protective (incomplete) antibodies which neutralize the aggressive enzyme of the anthrax bacillus.

Classification (see p. 393, Table 27).

Resistance. *B. anthracis* survives in meat-broth cultures in hermetically sealed ampoules for 40 years and the spores survive for 58 to 65 years. Spores remain viable in soil for decades and when dried—for as long as 28 years. They are more resistant to disinfectants than the vegetative cells. The vegetative cells are killed in 40 minutes at 55°C, in 15 minutes at 60°C, and in 1-2 minutes at 100°C. The spores are thermoresistant, and withstand boiling for 15-20 minutes and autoclaving at 110°C for 5-10 minutes. They are destroyed by 1 per cent formalin and 10 per cent sodium hydrate solutions in 2 hours. The capsule is more resistant than the microbial cell. Post-mortem examination of animal bodies which had

Table 27

Differentiation Between *B. anthracis*, Anthracoids, and Soil Bacilli

Bacilli	Motility	Capsule production	Growth characteristics		Pathogenicity for:		
			in blood broth	in litmus milk serum	mice	guinea pigs	rabbits
B. anthracis	—	+	No haemolysis	Turns red	Die in 24 hours	Die in 24-36 hours	Die in 36-72 hours
B. anthracoides	Slight	—	Haemolysis	Turns blue	Intraperitoneal injections of large amounts of culture are sometimes pathogenic	Nonpathogenic	
B. subtilis	Active	—	Haemolysis	Turns blue	Large doses of some strains are pathogenic for mice and guinea pigs. Rarely causes panophthalmia in man	Nonpathogenic	
B. megaterium	Moderate	—	No haemolysis	—		Nonpathogenic	
B. cereus var. mycoides	Slight	—	No haemolysis	—		Nonpathogenic	

where: + indicates motility and capsule production;
— indicates absence of motility and capsule production

previously been exposed to putrefactive microflora reveals quite frequently empty microbial capsules (shadows), devoid of cytoplasm.

Pathogenicity for animals. Sheep, cows, horses, deers, camels, and pigs are susceptible to anthrax infection. The animals are usually infected through the mouth, ingesting the spores in the fodder. The organisms localize in the intestines. Blood-sucking insects (gadflies and stable flies) may be vehicles of infection in some cases. The disease is characterized by lassitude, cyanosis, and sanguineous discharge from the intestines, nostrils, and mouth. Septicaemia sets in preceding death which occurs in 2 or 3 days. In horses the infection is less severe, affecting the glandular tissues and causing the development of anthrax carbuncles.

Among the laboratory animals, most susceptible are white mice, then guinea pigs, and rabbits which die 2-4 days after infection. A swelling and haemorrhages appear at the site of injection. The internal organs become congested and enlarged (particularly the spleen), and septicaemia develops. The blood of the animals does not clot after their death because of the anticoagulative effect of the anthrax bacilli, and it is thick and black-red in colour (Gr. *anthrax*—coal).

Pathogenesis and diseases in man. Anthrax is a typical zoonosis. Human beings acquire the disease from sick animals or articles and clothes manufactured from contaminated raw materials: sheepskin coats, fur mittens, collars, hats, shaving-brushes, etc. In summer the infection may be transmitted by blood-sucking insects. Anthrax occurs in three main clinical forms: cutaneous, respiratory, and intestinal.

In the cutaneous form the causative agent enters the body most frequently through injured integuments, mainly those parts of the body which are not covered with clothes (face, neck, hands, and forearms). An anthrax carbuncle (malignant pustule) develops at the site of bacilli localization. The disease prevails in individuals who come in contact with sick animals or raw materials contaminated with anthrax bacilli, and people who use articles manufactured from the hides or hair of infected animals.

The respiratory form is acquired through the air when working with material contaminated with bacillary spores. The disease assumes the form of a severe bronchopneumonia. The bacilli are discharged in the sputum.

The intestinal form is due to ingestion of meat of sick animals. It is characterized by grave lesions in the intestinal mucosa with haemorrhages and necrotic foci. The bacilli are excreted in the faeces. It is considered by a number of authors that the intestinal form of the disease is caused by the bacilli invading the intestines through the blood.

At present the cutaneous form of anthrax occurs sporadically, while the intestinal form is very rare. Due to labour protection

measures, cases of the respiratory form are extremely rare in the USSR.

Anthrax septicaemia may develop as a complication in any of the clinical forms or in debilitated and emaciated patients.

Immunity following anthrax is antimicrobial and depends on the presence of protective antigens. Phagocytosis plays no defensive role in the disease. The protective antigen does not give rise to the production of complete antibodies, but it stimulates the formation of protective (incomplete) antibodies which cause the destruction of the virulent anthrax bacilli.

Serum of individuals who have recovered from anthrax is found to contain substances which are capable of destroying capsular matter and neutralizing aggressins and toxins (lethal factor).

Laboratory diagnosis. In cases of cutaneous anthrax the malignant pustular exudate is examined; it is obtained from the deep layers of the oedematous area where it borders with the healthy tissues. Sputum is examined in cases of the respiratory form, faeces and urine, in intestinal form, and blood is examined in cases of septicaemia.

1. The specimens are examined under the microscope; the smears are Gram-stained, or stained by the Romanowsky-Giemsa method. The presence of morphologically characteristic capsulated bacilli, arranged in chains, allows a preliminary diagnosis.

2. For isolation of the pure culture the specimens are inoculated into meat-peptone agar and meat-peptone broth. The isolated culture is differentiated from other morphologically similar bacteria by its morphological and biochemical properties.

3. Laboratory animals (white mice, guinea pigs and rabbits) are inoculated with the pathological material and with the pure culture derived from it. *B. anthracis* causes the death of white mice in 24-48 hours and of guinea pigs in 2-3 days following inoculation. Microscopic examination of smears made from blood and internal organs reveals anthrax bacilli which are surrounded by a capsule.

A rapid biological test is also employed. The culture obtained which has to be identified is introduced intraperitoneally into white mice. Several hours after inoculation smears are prepared from the peritoneal contents. Detection of typical capsulated bacilli gives a basis for confirming the final result of the biological test.

The allergic test with anthracin (a purified anthrax allergen) is employed when a retrospective diagnosis is required in cases which have yielded negative results with microscopical and bacteriological examination. An intracutaneous injection of 0.05 ml of the preparation produces a noticeable reaction in 6 hours in positive cases, the final reaction being checked 24 hours after inoculation.

Post-mortem material as well as leather and fur used as raw materials are examined serologically by the thermoprecipitin reaction (Ascoli's test) since isolation of the bacilli is a matter of difficulty in such cases.

As can be seen in Fig. 106, the result in the first test tube (containing the test material) may be either positive or negative, in the second test tube (control) it must be only positive, and in the third, fourth, fifth, and sixth control test tubes the results must always be negative.

When employing laboratory diagnosis of anthrax, one must bear in mind the possibility of the presence of bacteria identical with *B. anthracis* in their biological properties (Table 27). These sporing aerobes are widely distributed in nature and are normally sporeforming saprophytes. They include *B. cereus* (Fig. 107), *B. subtilis*, *B. megaterium*, etc.

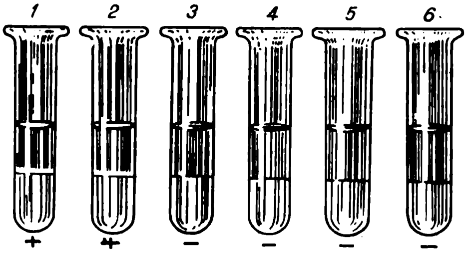


Fig. 106. Positive thermoprecipitation reaction (Ascoli's test)

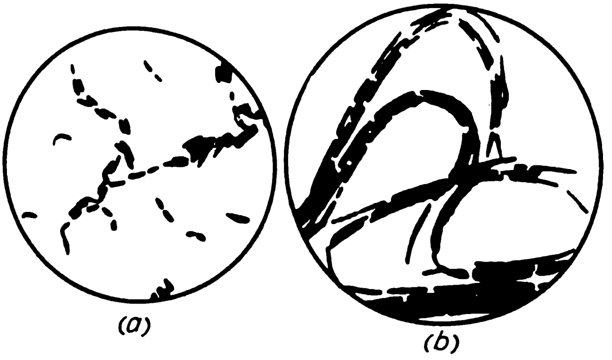


Fig. 107. *Bac. cereus*

a—the beginning of spore formation; *b*—impression smear from a 72-hour-old culture

The anthrax bacilli may be differentiated from anthracoids (false anthrax organisms) and other similar sporing aerobes by phagodiagnosis. The specific bacteriophage only causes lysis of the *B. anthracis* culture.

Treatment comprises timely intramuscular injection of antianthrax serum, and the use of antibiotics (penicillin, chlortetracycline, oxytetracycline, and streptomycin) and novarsenol.

Prophylaxis. General measures of anthrax control are carried out in joint action with veterinary workers. These measures are aimed at timely recognition, isolation, and treatment of sick animals. They also include thorough disinfection of premises for live-stock, territory and all objects found on it, and ploughing over of pastures. Carcasses of animals which have died of anthrax are burnt or buried on specially assigned territory, not less than 2 meters deep, and covered with lime chloride.

The veterinary authorities also enforce regulations banning the use of contaminated meat for food and introduce thorough control of manufactured articles from animal hide and fur which are to be marketed.

At present, a vaccine, prepared from noncapsulated anthrax bacilli and consisting of a suspension of live spores of vaccine strains, is used in the USSR. It is employed for immunization of human beings and domestic animals. The vaccine used for immunization by the skin method contains 4,000 million microbial cells per 1 ml, and the vaccine applied subcutaneously contains 100 million cells per 1 ml.

The vaccine is completely harmless, producing immunity quite rapidly (in 48 hours) and for a period of over a year. It is inoculated in a single dose.

Vaccination is carried out among people who work at raw-material processing factories (processing of animal hide and hair), at meat-packing factories, and at farms where anthrax is encountered. Re-inoculation is performed after a period of 12 months.

Individuals who have been in contact with material contaminated with anthrax organisms (when dressing infected carcasses or using such meat for food) are given intramuscular injections of 25, 50, or 100 ml of anthrax serum together with penicillin daily for 3-5 days.

A chemical anthrax vaccine consisting of a protective antigen (a filtrate of noncapsulated nonproteolytic anthrax strains which had been cultivated on synthetic and semisynthetic media) is used in England and the USA, it is as effective as the living vaccine.

The incidence of anthrax is rare in the USSR owing to the routine preventive measures carried out by the veterinary and medical services. During prerevolutionary times (1905-14) from 7,000 to 10,000 people suffered from anthrax every year in Russia. In the period between 1951 and 1956, 51,778 cases of anthrax were recorded in the other countries.

PATHOGENIC ANAEROBES

The pathogenic anaerobes belong to the order *Eubacteriales*, family *Bacillaceae*, and genus *Clostridium*. This group includes the causative agents of tetanus, gas gangrene, and botulism (Table 28), and the members have a number of features in common. These organisms are large Gram-positive bacilli which produce round- or oval-shaped terminal, central, or subterminal spores in the external environment. The spores are often considerably wider than the bacillus cell. The organisms grow under anaerobic conditions and produce exotoxins. They are always present in the intestines of man and animals and are excreted in the faeces. The spores remain viable in soil for long periods of time (for several years).

The causative agents of tetanus and gas gangrene are present in the intestines of man and ruminants as commensals, but when they penetrate damaged tissues (wounds) they produce severe and, not infrequently, fatal conditions.

TETANUS CLOSTRIDIA

A. Nicolaier discovered the causative agent of tetanus in 1884, and S. Kitasato isolated the pure culture in 1889.

Morphology. The causative agent of tetanus (*Clostridium tetani*) is a thin motile rod, 4-8 μ in length and 0.4-0.6 μ in breadth. It has peritrichous flagellation and contains granular inclusions which occur centrally and at the ends of the cell. The organism produces round terminal spores which give it the appearance of a drumstick (Fig. 108*a*). *Cl. tetani* is Gram-positive.

Cultivation. The organisms are obligate anaerobes. They grow on sugar and blood agar at pH 7.0-7.9 and at a temperature of 37°C (no growth occurs below 14° and above 43°C) and produce a pellicle with a compact center and thread-like outgrowths at the periphery. Sometimes a zone of haemolysis is produced around the colonies. Brain medium and bismuth-sulphite agar are blackened by *Cl.*

Table 2

General Characteristics of the Main Species of Pathogenic Anaerobes

Species	Clinical manifestations	Motility	Blood agar colonies	Growth in milk	Growth in gelatin	Changes in brain medium	Fermentation of carbohydrates									
							glycerin	mannitol	glucose	galactose	levulose	saccharose	lactose	maltose	inulin	[illegible]
Cl. tetani	Tetanus	Peritrichous	Two types of colonies: (1) fragile, lace-like, resemble small spiders (2) smooth, translucent, resemble dewdrops	Very slight coagulation	Slow liquefaction	Markedly blackened	—	—	—	—	—	—	—	—	—	-
Cl. perfringens	Gasgangrene	Nonmotile	Round succulent colonies, greyish at first, olive-coloured and green later, with zones of haemolysis	Rapid coagulation	Slow liquefaction	Not blackened	+	—	+	+	+	+	+	+	±	-
Cl. novyi (*Cl. oedematiens*)	Jelly-like serous oedema	Peritrichous	Rough, grey, with a raised centre, fringed edges, zones of haemolysis, and double-contoured filaments	Coagulation	Slow liquefaction	Not blackened	±	—	+	—	+	—	—	±	—	-
Cl. septicum	Serous-haemorrhagic oedema	Peritrichous	Fragile, lace-like, twisted threads; coils with zones of haemolysis	Coagulation	Slow liquefaction	Not blackened	—	—	+	+	+	—	+	+	—	-
Cl. histolyticum	Disintegration of tissues	Peritrichous	Small, smooth, resemble dew-drops, no haemolysis	Complete peptonization	Marked turbidity	Blackened slowly	—	—	—	—	—	—	—	—	—	-
Cl. botulinum	Botulism	Peritrichous	Similar to *Cl. novyi*	Complete peptonization	Liquefaction	Blackened slowly	+	—	+	—	—	—	—	+	—	-

where: + indicates carbohydrate fermentation with acid and gas formation; — indicates absence of fermentation

tetani. Agar stab cultures resemble a fir-tree or a small brush and produce fragile colonies which have the appearance of tufts of cotton wool or clouds (Fig. 108*b*). A uniform turbidity is produced on Kitt-Tarozzi medium with liberation of gas and a peculiar odour as a result of proteolysis.

Fermentative properties. *Cl. tetani* causes slow gelatin liquefaction and produces no indole. Nitrates are rapidly reduced to nitrites. The organisms coagulate milk slowly, forming small flakes. No carbohydrates are fermented.

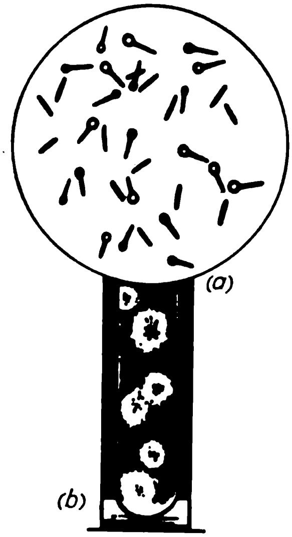


Fig. 108. *Clostridium tetani* with terminal spores (*a*); colonies (*b*)

Toxin production. *Cl. tetani* produces an extremely potent exotoxin which consists of two fractions; tetanospasmin, which causes muscle contraction, and tetanolysin, which haemolyses erythrocytes.

A 0.000005 ml dose of toxin obtained from a broth culture filtrate kills a white mouse which weighs 20g; and 0.00000005 g of dry toxin obtained by ammonium sulphate precipitation is fatal to the mouse. Several million lethal mouse doses are contained in 1 mg of crystalline toxin.

The mode of action of the tetanus toxin is similar to that of enzymes which catalyse chemical reactions in the bodies of affected animals.

Antigenic structure and classification. *Cl. tetani* is not serologically homogeneous and 10 serological types have been recognized. All 10 types produce the same exotoxin. The I, III, VI, and VII types exhibit a manifest specificity. The motile strains contain the H-antigen, and the nonmotile strains contain only the O-antigen. Type specificity is associated with the H-antigen and group specificity, with the O-antigen.

Resistance. Vegetative cells of the tetanus organism withstand a temperature of 60-70°C for 30 minutes and are destroyed quite rapidly by all commonly used disinfectants. The spores are very resistant, and survive in soil and on various objects over a long period of time and withstand boiling for 10-90 minutes or even, as with spores of certain strains, for 1-3 hours. The spores are killed by exposure to a 5 per cent phenol solution for 8-10 hours, and by a 1 per cent formalin solution, for 6 hours. Direct sunlight destroys them in 3-5 days.

Pathogenicity for animals. Horses and small cattle acquire the disease naturally, and many animals may act as carriers of *Cl. tetani*.

Among experimental animals, white mice, guinea pigs, rats, rabbits, and hamsters are susceptible to tetanus.

The disease in animals is manifested by tonic contractions of the striated muscles and lesions in the pyramid cells of the anterior cornua of the spinal cord. The extremities are the first to be involved in the process, the trunk being affected later (ascending tetanus).

Pathogenesis and disease in man. Sick people and animals, who discharge the organisms in their faeces into the soil, are sources of the infection. Spores of *Cl. tetani* can be demonstrated in 50-80 per cent of examined soil specimens, and some soils contain the spores in all test samples (manured soil is particularly rich in spores). The spores may be spread in dust, carried into houses, and fall on clothes, underwear, footwear, and other objects.

The majority of tetanus cases in adults occur among agricultural workers, and more than 33 per cent of the total incidence of the disease is associated with children from 1 to 15 years old. In more than 50 per cent of cases tetanus is acquired as the result of wounds of the lower extremities inflicted by spades, nails, and stubbles during work in the orchard or in the field.

Cl. tetani may gain entrance into the body of a newborn infant through the umbilical cord and into a woman during childbirth, through the injured uterine mucosa.

The organisms produce exotoxins (tetanospasmin and tetanolysin) at the site of entry. In some cases tetanus is accompanied by bacteraemia.

Microbes and spores, washed-off from the toxin, normally produce no disease and are rapidly destroyed by phagocytes.

The tetanus toxin reaches the motor centres of the spinal cord via the peripheral nerves (it moves along the axial nerve cylinders or along the ecto- and endoneural lymphatics).

According to the school of thought of A. Speransky, the specificity of the tetanus toxin is manifest only at the onset of the disease. In its further stages the infection is governed by other phenomena, primarily by the neurodystrophic factors. Sites of high and increasing excitation develop under the influence of irritation stimuli.

The toxin enters the blood and is thus distributed throughout the whole body, causing subsequent excitation of the peripheral nerve branches and the cells of the anterior cornua of the spinal cord.

Receptors situated in the neuromuscular apparatus play a significant role in the development of tetanus. Impulses sent out from these receptors give rise to a dominant excitation focus in the central nervous system. The effect of the toxin produces an increased reflex excitation of the motor centres, and this, in its turn, leads to the development of attacks of reflex tonic muscular spasms which may occur often in response to any stimuli coming from the external environment (light, sound, etc.).

The onset of the disease is characterized by persistent tonic muscular spasms at the site of penetration of the causative agent. This is followed by tonic spasms of the jaw muscles (trismus), face muscles (risus sardonicus), and occipital muscles. After this the muscles of the back (opisthotonos) and extremities are affected. Such is the development of the clinical picture of descending tetanus. The patient lies in bed, resting on his head and hips with his body bent forward like an arc. Death results from asphyxia and lesions of the vital centres. The death rate varies from 30 to 50 per cent, being 40 per cent on the average.

Immunity following tetanus is mainly antitoxic in character, and of low grade. Re-infections may occur.

Laboratory diagnosis is usually not carried out because clinical symptoms of the disease are self-evident. Objects of epidemiological significance (soil, dust, dressings, preparations used for parenteral injections) are examined systematically.

Wounds, dressings, and medicaments used for parenteral injections are examined for the presence of *Cl. tetani* and their spores by the following procedures. Specimens are inoculated into flasks or test tubes. The sowings are kept at a temperature of 80°C for 20 minutes to suppress the growth of any nonsporeforming microflora which may be present. After 2-10 days' incubation at 35°C, the culture is studied microscopically and tested for the presence of toxin by injection into mice. If *Cl. tetani* is present, tetanus of the tail develops during 24-48 hours, followed by tetanus of the body and death. The disease does not occur in mice which have been inoculated with antitetanic serum.

If no tetanus toxin is detected in the first inoculation but microscopic examination reveals the presence of organisms morphologically identical with *Cl. tetani*, the initial culture is inoculated into a concentrated aqueous solution of coagulated serum. A thin film will appear over the entire surface of the medium after 24 hours' growth in anaerobic conditions. Experimental animals are infected with a culture grown on liquid nutrient medium and kept for 4-5 days at 35°C.

A biological test is employed for detecting the exotoxin in the test material extract. Two white mice are given intramuscular injections of 0.5-1.0 ml of a centrifuged pricipitate or filtrate of the extract. An equal amount of the filtrate is mixed with antitoxic serum, left to stand for 40 minutes at room temperature, and then injected into another two mice in a dose of 0.75 or 1.5 ml per mouse. If the toxin is present in the filtrate, the first two mice will die in 2-4 days, while the other two (control mice) will survive.

Treatment. Intramuscular injections of large doses of antitoxic antitetanus serum (100,000-200,000 antitoxic units) are employed. Anticonvulsant therapy includes intramuscular injections of 25 per cent solutions of magnesium sulphate, administration of diplacine,

condelphine, aminazine, pipalphen or adaxine and chloral hydrate introduced in enemas. Together with serum therapy, 2 ml of toxoid is applied two hours before injecting the serum; the same dose of toxoid is repeated within 5-6 days. Uninoculated persons are subjected to active and passive immunization. This is achieved by injecting 0.5 ml of toxoid and 3,000 units of antitoxic serum and then 5 days later, another 0.5 ml of toxoid. The tetanus antitoxin is also introduced into previously inoculated individuals suffering from a severe wound. Injection of the total dose of antitoxin is preceded by an intracutaneous test for body sensitivity to horse protein. This is carried out by introducing 0.1 ml of antitoxin, previously diluted 1 : 100, into the front part of the forearm. If the intracutaneous test proves negative, 0.1 ml of whole antitoxin is injected subcutaneously and if no reaction is produced in 30 minutes, the total immunization dose is introduced. If another injury of a mild character occurs within 14 days following the first immunization, the injection of tetanus antitoxin is not repeated.

The complex of prophylactic measures includes adequate surgical treatment of wounds.

Prophylaxis is ensured by prevention of occupational injuries and traumas in everyday life.

Active immunization is achieved with tetanus toxoid. It is injected together with a tetravalent or polyvalent vaccine or may be a component of an associated adsorbed vaccine (see p. 260). The pertussis-diphtheria-tetanus vaccine and associated diphtheria-tetanus toxoid are employed for specific tetanus prophylaxis in children. Immunization is carried out among all children from 5-6 months to 12 years of age, individuals living in certain rural regions (in the presence of epidemiological indications), construction workers, persons working at timber, water-supply, cleansing and sanitation, and peat enterprises, and railway transport workers.

Immunization with tetanus toxoid stimulates the production of sufficient amounts of antitoxin. Immunity lasts for a period of 2 or 3 years.

CLOSTRIDIA RESPONSIBLE FOR GAS GANGRENE

Gas gangrene is a polybacterial infection. It is caused by several species of clostridia in association with various aerobic microorganisms (pathogenic staphylococci and streptococci).

The organisms responsible for gas gangrene are: (1) *Cl. perfringens*, (2) *Cl. novyi*, (3) *Cl. septicum*, and (4) *Cl. histolyticum*.

Cl. chauvoei, *Cl. fallax*, and *Cl. sporogenes* are pathogenic for animals.

Cl. aerofoetidum and *Cl. tertium* are nonpathogenic organisms which have significance in the pathogenesis of gangrene only in association with pathogenic bacteria.

Gas gangrene may be produced by any one of the first four species mentioned above but usually it is caused by several members of a parasitocoenosis acting in a particular combination. The less pathogenic and nonpathogenic species cannot be responsible for gas gangrene by themselves, but they cause tissue destruction, lower the oxidation-reduction potential, and thus create favourable conditions for the growth of pathogenic species.

CLOSTRIDIUM PERFRINGENS

The causative agent was discovered in 1892 by W. Welch and G. Nuttall. This organism occurs as a commensal in the intestines of man and animals. Outside of the host's body it survives for years in the form of spores. It is almost always found in the soil. The organism was isolated from 70-80 per cent of gas gangrene cases during World War I, and from 91-100 per cent of cases during World War II.

Morphology. *Cl. perfringens* is a thick pleomorphous nonmotile rod with rounded ends, 4-8 μ in length and 1-1.5 μ in breadth (Fig. 109). In the body of man and animals it is capsulated, and in nature

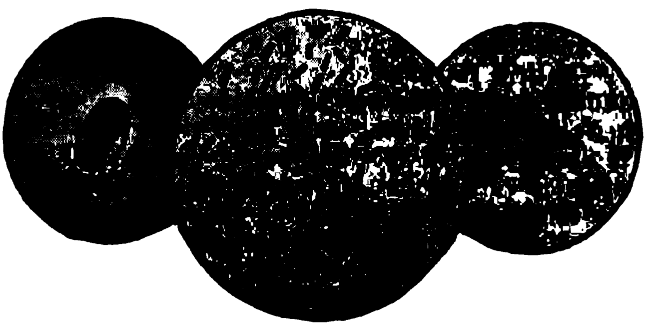


Fig. 109. Pure culture and colonies of *Clostridium perfringens*

it produces an oval, central or subterminal spore which is wider than the vegetative cell. *Cl. perfringens* stains readily with all aniline dyes and is Gram-positive but in old cultures it is usually Gram-negative.

Cultivation. *Cl. perfringens* is less anaerobic than the other causative agents of gas gangrene. It grows on all nutrient media which are used for cultivation of anaerobes. The optimum temperature for growth is 35-37°C (it does not grow below 16 and above 50°C), and optimal pH of medium is 6.0-8.0. A uniform turbidity and large volumes of gas are produced in cultures grown on Kitt-Tarozzi medium. Brain medium is not blackened. The colonies resemble discs or lentils deep in agar stab cultures (see Fig. 109). On blood

agar containing glucose smooth disc-like grey colonies are formed, with smooth edges and a raised centre.

Many strains of *Cl. perfringens* lose their anaerobic properties on exposure to antibiotics, bacteriophage, and X-rays and may be cultivated under aerobic conditions. Catalase and peroxidase, enzymes typically present in aerobic organisms, were revealed in the variants thus obtained. The aerobic variants are nontoxic and nonpathogenic for laboratory animals.

Fermentative properties. *Cl. perfringens* slowly liquefies gelatin, coagulated blood serum and egg albumen. The organism reduces nitrates to nitrites and normally no indole or only traces are produced. Volatile amines, aldehydes, ketones, and acetyl methyl carbinol are produced. Milk is vigorously coagulated and a sponge-like clot is formed. In meat medium the organism yields butyric and acetic acids and large quantities of gases (CO_2, H_2, H_2S, NH_3). It ferments glucose, levulose, galactose, maltose, saccharose, lactose, starch, and glycogen with acid and gas formation. Mannitol is not fermented.

Toxin production. The organism produces a toxin which has a complex chemical structure (lethal toxin, haemotoxin, neurotoxin, and necrotic toxin). It also produces lecithinase C which possesses enzymatic properties and splits lecithin into phosphorylcholine and diglyceride. Lecithinase is identified with alpha-toxin. In addition *Cl. perfringens* produces proteinase, fibrinolysin, collagenase, hyaluronidase, gelatinase, and desoxyribonuclease. Lecithinase C acts as a digestant enzyme in the intestines of man.

Due to such a complex of toxic substances and enzymes *Cl. perfringens* is capable of causing rapid and complete necrosis of muscular tissue. This process is the result of a combined effect of lecithinase, collagenase, and hyaluronidase on the muscles. Collagenase and hyaluronidase destroy the connective tissue of the muscles, and lecithinase C splits lecithin, a component in the muscle fibre membranes. Haemolysis in gas gangrene is due to the effect of lecithinase on lecithin of the erythrocyte stroma. The animal dies from rapidly developing asphyxia which is the result of intensive erythrocyte destruction and disturbances of the nerve centres.

Antigenic structure and classification. Six types of *Cl. perfringens* are distinguished: A, B, C, D, E, and F. These types are differentiated by their serological properties and specific toxins.

Type A is commonly found as a commensal in the human intestines, but it produces gas gangrene when it penetrates into the body by the parenteral route. Type B is responsible for dysentery in lambs and other animals. Type C causes haemorrhagic enterotoxaemia in sheep, goats, sucking pigs, and calves. Type D is the cause of infectious enterotoxaemia in man and animals, and type E causes enterotoxaemia in lambs and calves. Type F is responsible for human necrotic enteritis.

Resistance. The spores withstand boiling for a period of 8 to 90 minutes. The vegetative forms are most susceptible to hydrogen peroxide, silver ammonia, and phenol in concentrations commonly employed for disinfection.

Pathogenicity for animals. Among laboratory animals, guinea pigs, rabbits, pigeons, and mice are most susceptible to infection. Post-mortem examination of infected animals reveals oedema and tissue necrosis with gas accumulation at the site of penetration of the organism. Most frequently clostridia are found in the blood.

CLOSTRIDIUM NOVYI (CL. OEDEMATIENS)

The organism was discovered by F. Novy in 1894. Its role in the aetiology of gas gangrene was shown in 1915 by M. Weinberg and P. Seguin. It ranks second among the causative agents of gas gangrene. Soil examination reveals the presence of the organism in 64 per cent of the cases.

Morphology. *Cl. novyi* is a large pleomorphous rod with rounded ends, 5-14μ in length and 0.8-2μ in width, and occurs often in short chains (Fig. 110). The organism is motile, peritrichous, and may possess as many as 20 flagella. It forms oval, normally subterminal spores in the external environment. In the body of man and animals it is noncapsulated. The organism is Gram-positive.

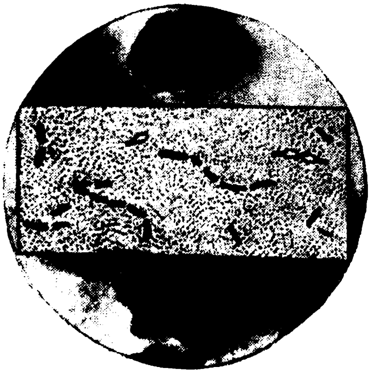


Fig. 110. Culture and deep colonies of *Clostridium novyi*

Cultivation. *Cl. novyi* is the strictest of the anaerobes. Its optimal growth temperature is 35-38°C (growth temperature ranges from 16 to 50°C), and optimal pH of medium is 7.8. Growth on Kitt-Tarozzi medium is accompanied by gas accumulation, precipitation, and clearance of the medium. On sugar-blood agar the colonies are rough, raised in the centre, and have fringed edges surrounded by zones of haemolysis. In agar stab cultures the organisms produce flocculent colonies with a dense centre from which thin filaments grow outwards.

Fermentative properties. The organisms slowly liquefy and blacken gelatin. They coagulate milk, producing small flakes. Glucose, maltose, and glycerin are fermented with acid and gas formation. Acetic, butyric, and lactic acids as well as aldehydes and alcohols are evolved as a result of the breakdown of carbohydrates.

Toxin production. *Cl. novyi* produces a potent exotoxin which is more stable than the toxin produced by *Cl. perfringens*. An active haemolysin possessing lecithinase properties is produced in *Cl. novyi* cultures.

Antigenic structure and classification. *Cl. novyi* is differentiated into four types: A, B, C, and D. Type A is responsible for gas gangrene in man, and type B causes infectious hepatitis, known as the black disease of sheep. Type C produces bacillary osteomyelitis in buffaloes, and type D is responsible for haemoglobinuria in calves.

Resistance. Spores survive in nature for a period of 20-25 years without losing their virulence. Direct sunlight kills them in 24 hours, boiling destroys them in 10-15 minutes. Spores withstand exposure to a 3 per cent formalin solution for 10 minutes. Coal-tar is an extremely active disinfectant.

Pathogenicity for animals. *Cl. novyi* causes necrotic hepatitis (black disease) in sheep. In association with nonpathogenic clostridia it produces bradsot (acute haemorrhagic inflammation of the mucous membranes of the true stomach and duodenum, accompanied by formation of gases in the alimentary canal and necrotic lesions in the liver) and haemoglobinuria in calves.

A subcutaneous injection of the culture into rabbits, white mice, guinea pigs, and pigeons results in a jelly-like oedema usually without the formation of gas bubbles. Post-mortem examination displays slight changes in the muscles; the oedematous tissues are pallid or slightly hyperaemic.

CLOSTRIDIUM SEPTICUM

The organism was isolated from the blood of a cow in 1877 by L. Pasteur and J. Joubert. In 1881 R. Koch proved the organism to be responsible for malignant oedema. It is found in 8 per cent of examined soil specimens.

Morphology. The clostridia are pleomorphous and may be from 3 to 8 μ long and from 0.6 to 0.8 μ thick; filamentous forms, measuring up to 50 μ in length, also occur (Fig. 111). The organisms are motile, peritrichous, and produce no capsules in the animal body. The spores are central or subterminal. The clostridia are Gram-positive but Gram-negative organisms occur in old cultures.

Cultivation. *Cl. septicum* are strict anaerobes. Their optimal growth temperature is 37°C, and they do not grow below 16°C. The pH of medium is 7.6. The organisms grow readily in meat-peptone broth and meat-peptone agar to which 5 per cent glucose has been added. On glucose-blood agar they produce a continuous thin film of intricately interwoven filaments lying against a background of haemolyzed medium. In agar stab cultures the colonies resemble balls of wool. In broth a uniform turbidity is produced, and an abundant loose, whitish, and mucilaginous precipitate later develops.

Fermentative properties. *Cl. septicum* liquefies gelatin slowly, produces no indole, reduces nitrates to nitrites, and decomposes proteins, with hydrogen sulphide and ammonia formation. Forcemeat is reddened but not digested; the culture evolving a rancid odour. Levulose, glucose, galactose, maltose, lactose, and salicin are fermented with acid and gas formation. Milk is coagulated slowly.

Toxin production. *Cl. septicum* produces a lethal exotoxin, necrotic toxin, haemotoxin, hyaluronidase, desoxyribonuclease, and collagenase. The organism haemolyses human, horse, sheep, rabbit, and guinea pig erythrocytes.

Antigenic structure and classification. On the basis of the agglutination reaction, 4 varieties of *Cl. septicum* can be distinguished.

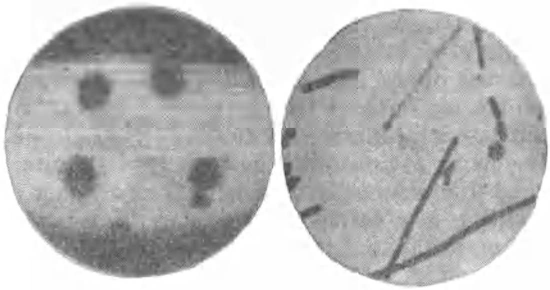


Fig. 111. Culture and deep colonies of *Clostridium septicum*

All four varieties produce identical toxins, the differential properties being associated with the structure of the H-antigen. *Cl. septicum* possesses antigens common to *Cl. chauvoei* which is responsible for gas gangrene in animals.

Resistance is similar to that of *Cl. novyi*.

Pathogenicity for animals. Among domestic animals horses, sheep, pigs, and cattle may contract the disease. Infected guinea pigs die in 18-48 hours. Post-mortem examination reveals crepitant haemorrhagic oedema and congested internal organs. The affected muscles have a moist appearance and are light brown in colour. Long curved filaments which consist of clostridia are found in impression smears of microscopical sections of the liver (see Fig. 111).

CLOSTRIDIUM HISTOLYTICUM

The organism was isolated in 1916 by M. Weinberg and P. Seguin. It produces fibrinolysin, a proteolytic enzyme, which causes lysis of the tissues in the infected body. An intravenous injection of the

exotoxin into an animal is followed shortly by death. The fact that the organisms are pathogenic for human beings has not met with general acceptance in the recent years. The organism's responsibility for gas gangrene during World War II was insignificant.

The main properties of the pathogenic types of clostridia are given in Table 28.

Pathogenesis and diseases in man. Gas gangrene is characterized by a varied clinical picture, depending on a number of factors. These include the number of pathogenic anaerobic species and their concomitant microflora, i.e., nonpathogenic or conditionally pathogenic anaerobes and aerobes which occur in particular associations reflecting the complex process of parasitocoenosis. The type of wound and the immunobiological condition of the body are also among the factors.

The causative agents of gas gangrene require certain conditions for their development after they have gained entrance into the body; i.e., favourable medium (the presence of dead or injured tissues) and a low oxidation-reduction potential (state of anaerobiosis) which arises due to the presence of necrotized cells of the affected tissues and aerobic microflora. Later the pathogenic anaerobes cause the necrosis of the healthy tissues themselves.

This process develops particularly intensively in the muscles owing to the fact that they contain large amounts of glycogen which serves as a favourable medium for pathogenic anaerobes responsible for gas gangrene. Oedema is produced during the first phase of the infection, and gangrene of the muscles and connective tissue, during the second phase.

The exotoxins which are produced by the gas gangrene clostridia exert not only a local effect, causing destruction of muscular and connective tissues, but affect the entire body. This results in severe toxaemia. The body is attacked also by toxic substances produced by the decaying tissues. Investigations have shown that exotoxins produced by the causative agents of gas gangrene possess potentiation activity. Simultaneous injections of one-fourth of a lethal dose of both *Cl. perfringens* and *Cl. novyi* toxins produce a reaction which is more marked than that produced by separate injections of the toxins into different parts of the body.

As a result of the vasoconstrictive effect of the toxins, development of oedema, and gas formation, the skin becomes pale and glistening at first and bronze-coloured later. The temperature of the affected tissues is always lower than that of the healthy areas. Deep changes occur in the subcutaneous cellular, muscle, and connective tissues, and degenerative changes take place in the internal organs.

The organisms themselves play an essential part in the pathogenesis of gas gangrene owing to their high invasive activity. An extremely important role in the development of the disease is attributed

to the reactivity state of the macroorganism (trauma, concomitant diseases, etc.).

M. Weinberg distinguished 3 main clinical forms of gas gangrene.

1. The emphysematous form which is characterized by abundant gas formation, the gas rapidly spreading in the tissues surrounding the wound. The necrotized tissues assume a greyish-green colour and discharge an odourless foamy fluid on being pressed. *Cl. perfringens* and *Cl. septicum* are isolated from cases with this form of the disease.

2. The oedematous-toxic form (N. Pirogoff named it white erysipelas) which is characterized by rapid development of oedema, marked paleness of the skin and severe intoxication. *Cl. novyi* are prevalent in the wounds.

3. The mixed form which is characterized by oedema and gas formation. A great number of associations of anaerobic and aerobic organisms are responsible for this form.

Ingestion of food (sheep's milk cheese, milk, curds, sausages, cod, etc.) contaminated abundantly with *Cl. perfringens* results in toxinfections and intoxications. These conditions are characterized by a short incubation period (from 2 to 6 hours), vomiting, diarrhoea, headache, chills, heart failure, and cramps in the gastrocnemius muscle; the body temperature may either be normal or elevated to 38°C.

Immunity. The immunity produced by gas gangrene is associated mainly with the presence of antitoxins which act against the most commonly occurring causative agents of the wound infection. For example, *Cl. perfringens* loses its lecithinase activity completely in the presence of a sufficient amount of antitoxin against its alpha-toxin.

The toxin-antitoxin reaction depends to a great extent on the presence of lecithin which acts as a substratum for toxin activity. The antitoxin cannot neutralize lecithinase if the former is added at certain periods of time after the toxin had been in the presence of lecithin, the reaction being simply somewhat delayed in such cases. A definite role is played by the antibacterial factor, since the existence of bacteraemia in the pathogenesis of gas gangrene has been shown.

Laboratory diagnosis. Material selected for examination includes pieces of affected and necrotic tissues, oedematous fluid, dressings, surgical silk, catgut, clothes, soil, etc. The specimens are examined in stages:

(1) microscopic examination of the wound discharge for the presence of *Cl. perfringens*;

(2) isolation of the pure culture and its identification according to the morphological characteristics of clostridia, capsule production, motility, milk coagulation, growth on iron-sulphite agar, gelatin liquefaction, and fermentation of carbohydrates (see Table 28);

(3) inoculation of white mice with broth culture filtrates or patient's blood for toxin detection;

(4) performance of the antitoxin-toxin neutralization reaction on white mice (a rapid diagnostic method).

Treatment and prophylaxis comprise the following procedures: (1) surgical treatment of wounds; (2) early prophylactic injection of a polyvalent purified and concentrated antitoxin "Diaferm 3" in a dose of 10,000 units against *Cl. perfringens*, 15,000 units against *Cl. novyi*, and 5,000 units against *Cl. septicum*. For treatment the doses of antitoxin are increased five-fold; (3) use of antibiotics (streptomycin, penicillin, synthomycin, chlortetracycline, and gramicidin), sulphonamides, anaerobic bacteriophages, and diphage. In a number of cases treatment with antitoxin alone does not give the desired effect, while the combined use of antitoxin and antibiotics significantly lowers the mortality rate.

Active vaccination is performed with tritoxoid, tetratoxoid, pentatoxoid (gas gangrene, tetanus, and botulism type A and B toxoids), and polytoxoid which contains gas gangrene, tetanus, and botulism A, B, C, and E toxoids.

CLOSTRIDIA RESPONSIBLE FOR BOTULISM

The causative agent of botulism (L. *botulus*—sausage, botulism—poisoning by sausage toxin), *Clostridium botulinum*, was discovered in Holland in 1896 by E. van Ermengem. The organism was isolated from ham which had been the source of infection of 34 people and from the intestines and spleen on post-mortem examination. In Western Europe botulism was due to ingestion of sausages, while in America it was caused by canned vegetables, and in Russia, by ingestion of red fish.

Morphology. *Cl. botulinum* is a large pleomorphous rod with rounded ends, 3-8 μ in length and 0.5-0.8 μ in breadth. The organism sometimes occurs in short forms or long threads. *Cl. botulinum* is slightly motile and produces from 4 to 30 flagella per cell. In the external environment *Cl. botulinum* produces oval terminal or subterminal spores which give them the appearance of tennis rackets (Fig. 112). The organisms are Gram-positive.

Cultivation. *Cl. botulinum* are strict anaerobes. The optimal growth temperature for types A, B, C, and D is 34-35°C, and for type E, 25-28°C (temperature limits 18-37°C). They grow on all ordinary media at pH 7.3-7.6. Cultivation is best on minced meat or brain which the organisms turn darker. The cultures have an odour of rancid butter.

On Zeissler's sugar-blood agar irregular colonies are produced which possess filaments or thin thread-like outgrowths. The colonies are surrounded by a zone of haemolysis.

In agar stab cultures the colonies resemble balls of cotton wool or compact clusters with thread-like filaments.

On gelatin the organisms form round translucent colonies surrounded by small areas of liquefaction. Later the colonies turn turbid, brownish, and produce thorn-like filaments.

In liver broth (Kitt-Tarozzi medium) turbidity is produced at first, but a compact precipitate forms later, and the fluid clears.

Fermentative properties. *Cl. botulinum* (types A and B) are proteolytic organisms, and decompose pieces of tissues and egg albumin in fluid medium. The organisms liquefy gelatin, produce hydrogen sulphide, ammonia, volatile amines, ketones, alcohols, and acetic, butyric, and lactic acids. Milk is peptonized with gas forma-

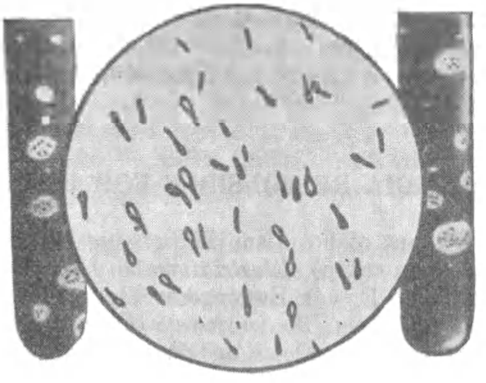


Fig. 112. Culture and colonies of *Clostridium botulinum*

tion. Glucose, levulose, maltose, and glycerin are fermented, with acid and gas formation.

Toxin production. *Cl. botulinum* produces an extremely potent exotoxin. The toxin is produced in cultures and foodstuffs (meat, fish, and vegetables) under favourable conditions and in the body of man and animals.

Multiplication of the organism and toxin accumulation are inhibited in the presence of a 6-8 per cent concentration of common salt or in media with an acid reaction. Heating at 90°C for 40 minutes or boiling for 10 minutes destroys the toxin.

The toxin produced by *Cl. botulinum*, as distinct from the tetanus and diphtheria toxins, withstands exposure to gastric juice and is absorbed intact. The toxin produced by type A *Cl. botulinum* can kill 60,000 million mice having a total weight of 1,200,000 tons,

while 15 g of type D *Cl. botulinum* toxin is sufficient to poison the entire population of the world. The toxin has been obtained in crystalline form and is the most potent of all toxins known to date.

The botulinum toxin is a globulin and does not change on recrystallization. Its activity is similar to that of enzymes which catalyse chemical processes in the body of man and animals with formation of large amounts of toxic substances. These substances produce the clinical manifestations of poisoning.

Antigenic structure and classification. Five serological types of *Cl. botulinum* are known: A, B, C, D, and E, types A, B, and E being the most toxic. Each of the types is characterized by specific immunogenicity associated with the H-antigen and is neutralized by the corresponding antitoxin. Types C and D are responsible for neuro-paralytic lesions in animals. As has been recently proven, type C may produce diseases in man also. The O-antigen is common to all types.

Resistance. The vegetative forms of the organisms are killed in 30 minutes at 80°C, while the spores withstand boiling for periods from 90 minutes to 6 hours and survive 115°C for 5-40 minutes and 120°C, for 3-22 minutes. Spores remain viable in large pieces of meat and in large cans even after autoclaving for 15 minutes at 120°C. In 5 per cent phenol solutions they survive for up to 24 hours and in cultures they may live for a year.

Pathogenicity for animals. Horses, cattle, minks, birds, and among the laboratory animals, guinea pigs, white mice, cats, rabbits, and dogs are susceptible to the botulinum toxin.

Paralysis of the deglutitive, mastication, and motor muscles is usually produced in horses 3 days after infection. The mortality rate reaches 100 per cent. Botulism in bovine cattle is accompanied by bulbar paralysis, and in birds it causes limberneck and paresis of the legs.

Infection of guinea pigs results in muscular weakness which appears in 24 hours, followed by death in 3-4 days. Autopsy displays hyperaemia of the intestines, gastric flatulence, and a urinary bladder filled beyond capacity. White mice die on the second day after infection manifesting relaxed abdomen muscles and paresis of the hind limbs. Paralysis of the eye muscles, disturbances of accommodation, aphonia, pendulous and protruding tongue, and diarrhoea are caused in cats.

Pathogenesis and disease in man. Botulism is contracted by ingesting meat products, canned vegetables, sausages, ham, salted and smoked fish (red fish more frequently), canned fish, chicken and duck flesh, and other products contaminated with *Cl. botulinum.* The organisms enter the soil in the faeces of animals (horses, cattle, minks, and domestic and wild birds) and fish and survive there as spores.

Natural nidality of botulism among ducks and other wild birds has been ascertained. Extremely widespread epizootics occur in the western regions of Canada, in Uruguay, and in the USA. Natural foci develop in territories where there are stagnant reservoirs rich in vegetable debris which provides favourable conditions for anaerobiosis in warm weather. Intensive processes of decay are accompanied by oxygen uptake which contributes to the growth of type C botulism clostridia and production of high concentrations of the exotoxin in the water and alkaline mud in swamps. Besides birds, muskrats and frogs may also contract botulism. Migrating birds spread the causative agent of botulism from the natural foci during their flights.

Cl. botulinum spores occur both in cultivated and virgin soil. They were isolated from 70 per cent of examined soil samples in California, and from 40 per cent of samples in the Northern Caucasus. Spores have been isolated from littoral soil at the Sea of Azov, sea water, and silt. They have also been found on the surface of vegetables and fruit, in the intestines of healthy animals, in 5.4 per cent of cases in the intestines of fresh red fish (sturgeon, beluga, etc.), and in 15-20 per cent of cases in the intestines and in 20 per cent of cases in the tissues of dead fish.

The infectious condition is caused by the exotoxin which is absorbed in the intestines, from where it invades the blood, and affects the medulla oblongata nuclei, cardiovascular system, and muscles. It has been ascertained that *Cl. botulinum* may enter the body through wounds. In the past botulism was considered to be only of a toxic nature. Recent investigations have proved the *Cl. botulinum* to be present in various organs of individuals who have died from botulism. Therefore, this disease is a toxinfection.

Botulism symptoms include dizziness, headache, and, sometimes, vomiting. Paralysis of the eye muscles, accommodation disturbances, dilatation of the pupils, and double vision occur. Difficulty in swallowing, aphonia, and deafness also arise. The death rate is very high (40-60 per cent).

Immunity. The disease leaves a stable anti-infectious immunity (antitoxic and antibacterial).

Laboratory diagnosis. Remains of food which caused poisoning, blood, urine, vomit, faeces, and lavage waters are examined. Post-mortem examination of stomach contents, portions of the small and large intestines, lymph nodes, and the brain and spinal cord is carried out.

The test specimens are inoculated into Kitt-Tarozzi medium which has previously been held at 100°C for 10-20 minutes. To free the cultures from foreign nonsporeforming microflora, 50 per cent of the test tubes containing the inoculated medium are heated at 80°C for 20 minutes and then incubated in anaerobic conditions. The iso-

lated pure culture is identified by its cultural, biochemical, and toxigenic properties.

For toxin detection a broth culture filtrate, patient's blood or urine, or extracts of food remains, are injected subcutaneously or intraperitoneally into guinea pigs, white mice, or cats. One of the control animals is infected with unheated material, while the other animal is injected with the heated specimen. In addition, 3 laboratory animals are given injections of the filtrate together with type A antitoxin, with type B antitoxin, and with type E antitoxin.

The indirect haemagglutination reaction and determination of the phagocytic index are also performed. This index is significantly lowered in the presence of the toxin.

A rapid method of detection of type A, B, C, D, and E toxins in water has been elaborated in which the toxin is absorbed by talc and a suspension of the talc and toxin is injected into the animals.

Treatment. The stomach is lavaged with potassium permanganate or soda solutions. Polyvalent botulinum antitoxin (types A, B, and E) is injected intramuscularly (intravenously or into the spinal canal) in doses of 50,000-100,000 units. If there is no improvement, the injection is repeated at the same dosage within 5-10 hours. All individuals who had used food which caused even a single case of food poisoning are given 25,000-50,000 units of antitoxin as a preventive measure. Simultaneously with the antitoxin, 0.5 ml of each type of botulinum toxoid is injected three times at intervals of 3-5 days, for production of active immunity. Penicillin and tetracycline are recommended.

General measures include subcutaneous injections of saline and glucose solutions. Camphor, caffeine, vitamin C, and thiamine are prescribed if necessary. Strychnine is given 2-3 times a day as a stimulant.

Prophylaxis. Proper organization of food processing technology at food factories, meat, fish, and vegetable canning in particular, and preparation of smoked and salted fish and sausages is essential for the prevention of botulism. Home-preserved fish products (smoked and salted) are very dangerous since they are usually prepared without observance of sanitary rules.

Fish should be gutted after being caught, and placed in the refrigerator. The established temperature regime must be observed during transportation, and the fish must be protected from pollution with soil and bowel contents. Vegetables must be washed thoroughly. The cooking of meat and fish in small pieces is recommendable. Foodstuffs (ham, fish) should not be stored in large hunks and in many layers. The weight of a canned product should not exceed 0.5 kg. *Cl. botulinum* which have withstood sterilization cause swelling of the can lids. The contents of such cans have an odour of rancid butter. Such canned goods must not be put on the

market and must be withdrawn and thoroughly examined. Fish must be salted in strong salt solutions (brine) with a minimal concentration of 10 per cent. Canned goods must be stored in a cool place.

Active immunization of human beings, horses, and cows with the toxoid is recommended by many authors in view of *Cl. botulinum* being widespread in nature.

Botulism occurrence in the USSR is extremely rare as a result of continuous improvement of the standard of living of the population, improvement of technological methods in food processing and canning industries, observance of the rules of hygiene and strict state and sanitary control of the production, storage, and sale of foodstuffs.

PATHOGENIC CORYNEBACTERIA

The family *Corynebacteriaceae*, order *Eubacteriales*, consists of organisms which have terminal club-shaped swellings, e.g., the agent responsible for diphtheria (Gr. *diphthera*—skin, membrane), diphtheroids (nonpathogenic corynebacteria), listeria, and *Erysipelothrix*.

CAUSATIVE AGENT OF DIPHTHERIA

Extensive clinical, pathoanatomical, epidemiological, and experimental investigations preceded the discovery of the agent responsible for diphtheria. They paved the way for the discovery of the organism (E. Klebs, 1883), its isolation in pure culture (F. Loeffler, 1884), separation of the toxin (E. Roux and A. Yersin, 1888), antitoxin (E. Behring and S. Kitasato, 1890) and diphtheria toxoid (G. Ramon, 1923).

Morphology. *Corynebacterium diphtheriae* (L. *coryna*—club) is a straight or slightly curved rod, 18-μ in length and 0.3-0.8μ in breadth. The organism is pleomorphous and stains more intensely at its ends (see Figs. 5 and 117, *3*) which contain volutin granules (Babes-Ernst granules, metachromatin). *C. diphtheriae* frequently display terminal club-shaped swellings. Branched forms as well as short, almost coccal, forms sometimes occur. In smears the organisms are arranged at an angle and resemble spread-out fingers. They are Gram-positive and produce no spores, capsules, or flagella.

C. diphtheriae may change into cone-shaped, thread-like, fungi-like, and coccal forms (see Figs. 50 and 51). In old cultures the cytoplasm of the organisms acquires a zebra-like appearance with unequally stained stripes.

Cultivation. The causative agent of diphtheria is an aerobe or a facultative aerobe. The optimal temperature for growth is 34-37°C

and the organism does not grow at temperatures below 15 and above 40° C. The pH of medium is 7.2-7.6. The organism grows readily on media which contain protein (coagulated serum, blood agar, and serum agar) and on sugar broth. On Roux's (coagulated horse serum) and Loeffler's (three parts of ox serum and one part of sugar broth) media the organisms produce growth in 16-18 hours. The growth resembles shagreen leather, and the colonies do not merge together.

According to cultural and biological properties, 3 varieties of *C. diphtheriae* can be distinguished, *gravis*, *mitis*, and *intermedius*, which differ in a number of properties.

Corynebacteria of the *gravis* type produce large, rough (R-forms), rosette-like black or grey colonies (Fig. 113*a*) on tellurite agar which

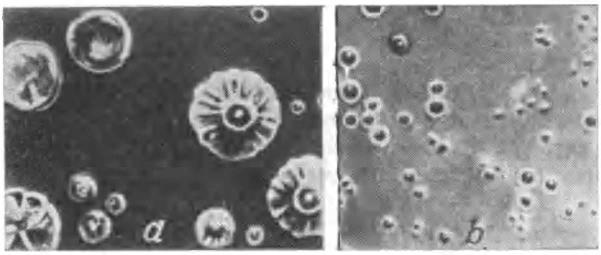


Fig. 113. Colonies of *Corynebacterium diphtheriae*
a—gravis, *b*—mitis

contains defibrinated blood and potassium tellurite. The organisms ferment dextrin, starch, and glycogen and produce a pellicle and a granular deposit in meat broth. They are usually highly toxic with very marked invasive properties.

The colonies produced by corynebacteria of the *mitis* type on tellurite agar are dark, smooth (S-forms), and shining (Fig. 113*b*). Starch and glycogen are not fermented, and dextrin fermentation is not a constant property. The organisms cause haemolysis of all animal erythrocytes and produce diffuse turbidity in meat broth. Cultures of this type are usually less toxic and invasive than those of the *gravis* type.

Organisms of the *intermedius* type are intermediate strains. They produce small (RS-forms) black colonies on tellurite agar. Starch and glycogen are not fermented. Growth in meat broth produces turbidity and a granular deposit.

Fermentative properties. All three types of *C. diphtheriae* do not coagulate milk, do not break down urea, produce no indole, and slowly produce hydrogen sulphide. They reduce nitrates to nitrites. Potassium tellurite is also reduced, and for this reason *C. diphtheriae* colonies grown on tellurite agar turn black or grey. Glucose and

levulose are fermented whereas galactose, maltose, starch, dextrin, and glycerin fermentation is variable. Exposure to factors in the external environment renders the organisms incapable of carbohydrate fermentation.

Toxin production. An extremely potent exotoxin is produced in meat broth cultures. Small doses of the toxin cause toxaemia in guinea pigs.

The diphtheria toxin contains large amounts of amino-nitrogen and catalyses chemical reactions in the body. The toxigenic strains of *C. diphtheriae* are characterized by marked dehydrogenase activity, while the nontoxigenic strains do not possess such activity.

The diphtheria toxin is unstable, and is destroyed easily by exposure to heat, light, and oxygen of the air, but is relatively resistant to supersonic vibrations. The toxin is transformed into the toxoid by mixture with 0.3-0.4 per cent formalin and maintenance at 38-40°C for a period of 3 or 4 weeks. The toxoid is more resistant to physical and chemical factors than the toxin.

Under the influence of a moderate bacteriophage of the *gravis* type, a lysogenic transformation may take place in some cases and, as a result, diphtheria corynebacteria of the *mitis* type become more toxigenic.

Antigenic structure. Several serological types of *C. diphtheriae* have been determined on the basis of the agglutination reaction. There is evidence that 57 serotypes of the organism exist. They all produce toxins which do not differ from each other and are neutralized completely by the standard diphtheria antitoxin. A number of authors have confirmed the presence of type-specific thermolabile surface protein antigens (K-antigens) and group-specific thermostable somatic polysaccharide antigens (O-antigens) in the diphtheria corynebacteria.

Classification. The order *Corynebacterium* comprises a species pathogenic for human beings and several species which are nonpathogenic for man and conditionally designated as diphtheroids. The majority of diphtheroids occurs in the external environment (water, soil, air), some of them are present as commensals in the human body, and certain diphtheroid strains are pathogenic for animals (*Corynebacterium enzymicum*). Properties of differentiation between diphtheria corynebacteria and the diphtheroids are given in Table 29. Toxin production is the most significant differential criteria. However, even this property is not reliable for identification purposes because there are *C. diphtheriae* strains which produce no exotoxin but, nevertheless, they may be responsible for diseases in human beings.

Resistance. *C. diphtheriae* are relatively resistant to harmful environmental factors. They survive for 1 year on coagulated serum, for 2 months at room temperature, and for several days on children's toys. Corynebacteria remain viable in the membranes of diphtheria

Table 29

Main Properties of Causative Agents of Diphtheria and Diphtheroids

Species	Pathogenicity for man and certain animals	Exotoxin production	Sheep erythrocyte haemolysis	Formation of volutin granules	Oxygen requirements	Gelatin liquefaction	Fermentation of: glucose	maltose	saccharose	mannitol	starch	urea	cystine
Corynebacterium diphtheriae t. gravis	+	+	±	+	Facultative aerobes	—	+	+	—	—	+	—	+
t. mitis	+	+	+	+		—	+	+	—	—	—	—	+
t. intermedius	+	+	—	+		—	+	+	—	—	—	—	+
Corynebacterium pseudodiphtheriticum	—	—	—	∓	Aerobe	—	—	—	—	—	—	+	—
Corynebacterium xerosis	—	—	—	∓	Facultative aerobe	—	+	+	+	—	—	—	—
Corynebacterium enzymicum	+	—	—	—	Facultative aerobe	+	+	+	+	—	—	?	
Corynebacterium pyogenes	—	+	+	—	Facultative aerobe	+	+	+	+	—	—	—	

patients for long periods, particularly when the membranes are not exposed to light. The organisms are killed by a temperature of 60° C and by a 1 per cent phenol solution in 10 minutes.

Pathogenicity for animals. Animals do not naturally acquire diphtheria. Although virulent diphtheria organisms were found to be present in horses, cows, and dogs, the epidemiological significance of animals in diphtheria is negligible.

Among the laboratory animals, guinea pigs and rabbits are most susceptible to the disease. Inoculation of these animals with a culture or toxin gives rise to typical manifestations of a toxinfection and the appearance of inflammation, oedema, and necrosis at the site of inoculation. The internal organs become conjested, particularly the adrenals in which haemorrhages occur.

Pathogenesis and disease in man. Patients suffering from the disease and carriers are the sources of infection in diphtheria. The disease is transmitted by an air-droplet route, and sometimes with dust particles. Transmission by various objects (toys, dishes, books, towels, handkerchiefs, etc.) and foodstuffs (milk, cold dishes, etc.) contaminated with *C. diphtheriae* is also possible.

Carriers play an essential part in the epidemiology of diphtheria. The carrier state averages from 3 to 5 per cent among convalescents and healthy individuals.

Diphtheria is most prevalent in the autumn. This is due to the fact that children are more crowded in the autumn months and that body resistance is reduced by a drop in temperature.

The exotoxin is of greatest importance in the pathogenesis of diphtheria. It has been established by many authors that *C. diphtherial* is capable of producing hyaluronidase and invading the blood and tissues of patients and infected animals. For this reason diphtheria is considered to be a toxinfection.

Clinical studies and experiments on animals have provided evidence of the influence of pathogenic staphylococci and streptococci on the development of diphtheria, the infection becoming more severe in the presence of these organisms. Nontoxic diphtheria corynebacteria as well as anaerobic corynebacteria may be responsible for some cases of diphtheria. Hypersensitivity to *C. diphtheriae* and to the products of their metabolism is of definite significance in the pathogenesis of diphtheria.

In human beings, membranes containing a large number of *C. diphtheriae* and other bacteria are formed at the site of entry of the causative agent (pharynx, nose, tracheae, eye conjunctiva, skin, vulva, vagina, and wounds). The toxin produces diphtherial inflammation and necrosis in the mucous membranes or skin. On being absorbed, the toxin affects the nerve cells, cardiac muscle, and parenchymatous organs and causes severe toxaemia.

Deep changes take place in the cardiac muscle, vessels, adrenals, and in the central and peripheral nervous systems.

According to the site of the lesion, faucial diphtheria and diphtheritic croup occur most frequently, and nasal diphtheria somewhat less frequently. The incidence of diphtheria of the eyes, ears, genital organs, and skin is relatively rare. Faucial diphtheria constitutes more than 90 per cent of all the diphtherial cases, and nasal diphtheria takes the second place.

Immunity following diphtheria depends mainly on the antitoxin content in the blood. However, a definite role of the antibacterial component, associated with phagocytosis and the presence of opsonins, agglutinins, precipitins, and complement-fixing substances cannot be ruled out. Therefore, immunity produced by diphtheria is anti-infectious (antitoxic and antibacterial) in character.

Schick test. This test is used for detecting the presence of antitoxin in children's blood. The toxin is injected intracutaneously into the forearm in a 0.2 ml volume which is equivalent to $^1/_{40}$ M.L.D. for guinea pigs. A positive reaction, which indicates susceptibility to the disease, is manifested by an erythematous swelling measuring 2 cm in diameter which appears at the site of injection in 24-48 hours. The Schick test is positive when the blood contains either no antitoxin or not more than 0.005 units per millilitre of blood serum. A negative Schick reaction indicates, to a certain degree, insusceptibility to diphtheria.

In newborn infants the Schick test is negative in 80-90 per cent of cases, in 9-12-month-old infants the percentage of positive reactions increases to 90, and among 16-17-year olds incidence of the negative reaction again reaches 80 per cent.

Children from 1 to 4 years old are most susceptible to diphtheria. A relative increase of the incidence of the disease among individuals 15 years of age and older has been noted in recent years.

Diphtheria leaves a less stable immunity than do other children's diseases (measles, whooping cough). Diphtheria reinfection occurs in 6-7 per cent of the cases.

Laboratory diagnosis. Discharges from the pharynx, nose, and, sometimes, from the vulva, eyes, and skin are collected with a sterile cotton-wool swab for examination.

The material under test is seeded on special media, e.g., coagulated serum, Clauberg's II medium, blood-tellurite agar, serum-tellurite agar, etc. Smears are examined under the microscope after 12-24-48-hours' growth, and preliminary diagnosis is made on the basis of microscopic findings.

C. diphtheriae does not always occur in its typical form. Short rods arranged not at a particular angle but in disorder and containing few granules are found in a number of cases. Diagnostic errors are made most frequently when investigations are confined to microscopical examination. Other bacterial species and nonpathogenic corynebacteria which are morphologically identical with the diphtheria organisms may be mistaken for the diphtheria corynebacte-

ria. It must also be borne in mind that formation of volutin granules is variable, and, therefore, this is not an absolute property. For this reason, contemporary laboratory diagnosis comprises isolation of the pure culture and its identification by cultural, biochemical, serological and toxigenic properties.

The toxigenic and nontoxigenic strains of diphtheria corynebacteria are differentiated either by subcutaneous or intracutaneous infection of guinea pigs, or by the agar precipitation method, the latter being relatively simple and may be carried out in any laboratory. It is based on the ability of the diphtheria toxin to react with the antitoxin (see p. 234) and produce a precipitate resembling arrow-tendrils (Fig. 114).

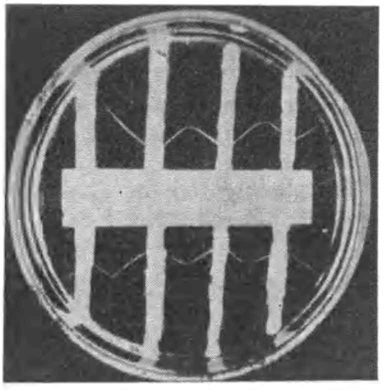


Fig. 114. Production of precipitins by toxigenic *C. diphtheriae* cultures

The agglutination reaction with patient's sera (similar to the Widal reaction) is employed as an auxiliary and retrospective method. It is performed with 5 serological types of *C. diphtheriae*; the reaction is considered positive beginning from 1:50-1:100 dilutions of serum.

Treatment. According to the physician's prescriptions, patients are given antitoxin in doses ranging from 10,000 to 50,000 units in mildly severe cases, and from 100,000 to 250,000 units in severe cases of the disease. Chlortetracycline, penicillin, streptomycin, oxytetracycline, erythromycin, sulphonamides, and cardiac drugs are also employed. Diphtheria toxoid is recommended in definite doses for improving the immunobiological state of the body, i. e., for stimulating antitoxin production.

Carriers are treated with antibiotics. Tetracycline, erythromycin, and oxytetracycline in combination with vitamin C are very effective.

Prophylaxis. General control measures comprise early diagnosis, prompt hospitalization, thorough disinfection of premises and objects, recognition of carriers, and systematic health education.

Specific prophylaxis is afforded by active immunization. A number of preparations are used: the pertussis-diphtheria vaccine, purified adsorbed toxoid, pertussis-diphtheria-tetanus vaccine, associated diphtheria-tetanus toxoid, and the diphtheria-pertussis-scarlatina vaccine. All preparations are used according to instructions and directions.

It should be noted that not all immunized children acquire resistance to diphtheria. An average of 5-10 per cent of them remain

susceptible or refractory (not capable of producing antibodies after immunization). Such a condition is considered to be the result of tolerance (see p. 220), agammaglobulinaemia, or hypoagammaglobulinaemia (see p. 218).

Contacts of diphtheria patients are given protective intramuscular injections of antitoxin in a 1,000-3,000 unit dose if no medical observation is possible. In such cases simultaneous immunization with the toxoid is employed.

Great success has been achieved in the control of diphtheria as a result of compulsory immunization against this severe children's disease. The task of complete abolishment of diphtheria within the next few years is being aimed at.

LISTERIA

The causative agent of listeriosis (*Listeria monocytogenes*) was discovered in 1926 by E. Murray and named *Listeria* in honour of J. Lister in 1940 by J. Pirie.

Morphology. Listeria are small bacteria (Fig. 115) 0.5-2μ in length and 0.4-0.5μ in breadth. They are motile, slightly curved, terminally flagellated and Gram-positive. The organisms occur singly or in pairs, and in smears from organs they are often seen arranged at an angle to each other in the form of the letter V or in chains. They do not form spores or capsules.

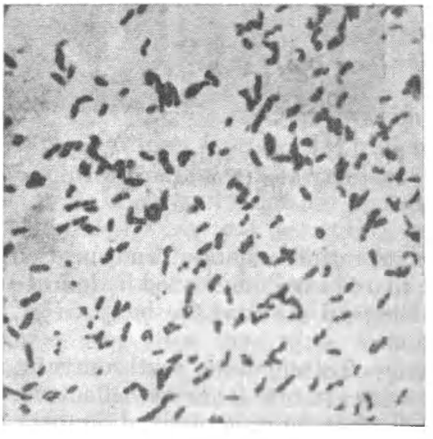


Fig. 115. Listeria

Cultivation. Listeria are facultative aerobes with simple growth requirements. They grow on all ordinary media at pH 7.0-7.2 and 37° C. No growth is shown below 2.5 and above 59° C. On solid nutrient media the organisms produce small, whitish, flat, smooth, and shiny colonies with a pearly hue. On liver agar the colonies are slimy. In broth listeria produce turbidity and a slimy deposit. On blood agar the colonies are surrounded by a narrow zone of haemolysis.

Listeria produce forms which are resistant to antibiotics as well as antibiotic-dependent varieties. The S-forms of the organisms are characterized by sensitivity to phagolysis, while the R-forms are phage resistant. Eight phage types can be distinguished on the basis of phagolysis.

Fermentative properties. Litmus milk turns red but is not coagulated. Listeria produce no indole or hydrogen sulphide, do not reduce nitrates to nitrites, and do not liquefy gelatin. Glucose, levulose, and trehalose are fermented with acid but no gas formation. Fermentation of maltose, lactose, saccharose, dextrin, salicin, rhamnose, and soluble starch is variable and slow.

Toxin production. The organisms produce no soluble toxin (exotoxin). An endotoxin is liberated when the bacterial cells disintegrate and is responsible for the characteristic manifestations of listeriosis in human beings and animals.

Antigenic structure and classification. There are two main serological types of listeria: rodent and ruminant. The former type was isolated from rodents, and is the most widespread. The latter type was isolated from ruminants (bovine cattle). However, this classification is only relative since both serological types have also been found in other animals, birds, and human beings. The main serotypes possess somatic (O) and flagellar (H) antigens. The somatic O-antigen contains four thermostable antigens (I, II, IV, and V) and one variable antigen (III). The H-antigens contain antigens A, B, C, and D which are destroyed by exposure to formalin.

Resistance. Listeria are resistant to environmental factors. They maintain their pathogenic properties in the dried state for a period of 7 years, and withstand freezing. They survive at 55° C for one hour and 58° C for 30 minutes. The organisms are killed in 3 minutes by boiling and in 20 minutes by a temperature of 70° C. They are destroyed by exposure to 1 and 0.5 per cent formalin solutions, 5 per cent phenol solution and to other disinfectants.

Pathogenicity for animals. Cattle, sheep and goats, horses, pigs, rabbits, chickens, and pigeons may naturally acquire the disease. The infection occurs among domestic and field mice and among wild rats which are probably the main reservoir of the causative agent in nature.

Rabbits, guinea pigs, and mice are most susceptible to the disease among the laboratory animals. Intracerebral inoculation results in sepsis which leads to death in 1 or 4 days. Protracted cases give rise to meningoencephalitis. The disease may also be brought about in laboratory animals by subcutaneous, intramuscular, and intranasal inoculation.

Pathogenesis and diseases in man. Listeriosis is a zoonotic infection. Human beings contract it from sick rodents, pigs, and horses. Meat products derived from pigs affected with listeriosis are most dangerous to man. Infection is possible through tick bites in enzootic listeriosis foci. The causative agent enters the body through injured skin, through the mucous membranes of the mouth, nasopharynx, and intestinal tract and through the eye conjunctiva. The diseases are characterized by sepsis (acute and chronic) and symptoms of meningoencephalitis which is fatal in most cases, particularly among

newborn infants and people with cerebral injuries. Inflammatory processes develop in the pharynx, and a skin rash sometimes appears. Apart from cases with severe clinical manifestations, mild forms of the disease and carrier states may occur.

Great significance in the pathogenesis of listeriosis is attributed to exotoxin saturation of the whole body or of separate tissues and organs, the causative agent multiplying intensively in the body of infected man or animals.

The liver, spleen, lymph nodes, heart, central nervous system, meninges, uterus, and the internal organs of newborn infants are the most seriously involved.

There are two main forms of human listeriosis: anginose-septicaemic and nervous. Recovery from the former is normally possible, but death may occur in some cases with both forms. Septicogranulomatous (in foetus and newborn infants) and ocular-glandular forms occur in human beings besides the two above mentioned main forms.

Immunity. Animals acquire immunity following listeriosis, regardless of the presence of a reservoir of the causative agent (infected rats and ticks). Immunity in man has not been studied sufficiently. Agglutinins, precipitins, and complement-fixing antibodies have been found to be present in patients' blood, but they do not show antibacterial effect in laboratory tests. Hyperimmune serum has no therapeutic properties. A rise in antibody titre is used in laboratory diagnosis.

Laboratory diagnosis is performed by isolating listeria cultures from the patients' blood. Specimens of brain tissue, pieces of liver and spleen are collected for examination at autopsy. The best growth is obtained in glucose-serum broth. In addition, laboratory animals are infected.

Serological diagnosis comprises the agglutination reaction which is positive in patients' sera diluted in ratios ranging from 1:250 to 1:5,000. The precipitin reaction and the complement-fixation reaction are also employed.

When identifying listeria, it is necessary to differentiate them from the organisms responsible for swine erysipelas. These organisms differ from listeria in that they are nonmotile, incapable of fermenting salicin, and nonpathogenic for guinea pigs. The antigenic structure of both organisms is different and strictly specific.

Treatment is accomplished by the use of symptomatic drugs, antibacterial preparations of the chlortetracycline group, and sulphonamides.

Prophylaxis is ensured by general sanitary measures carried out jointly with veterinary service. Laboratory control of meat which is to be marketed, routine control over domestic animals, timely recognition of rodent enzootics, and prevention of horses being infected by affected rodents and domestic animals are all necessary.

CAUSATIVE AGENT OF ERYSIPELOID

The organism responsible for erysipeloid (*Erysipelothrix insidiosa*) was isolated from the skin of patients in 1884-86 by F. Rosenbach.

Morphology. The organism is a nonmotile, slender or slightly curved rod (Fig. 116). It is pleomorphous, Gram-positive, 0.5-2.5μ in length and 0.2-0.4μ in breadth. The organism produces no spores or capsules.

Cultivation. The erysipeloid organisms grow readily on all common nutrient media at pH 7.4-7.8 and 37° C (growth temperatures range from 16 to 41° C) and are facultative aerobes. On solid media they produce fragile, small, and translucent colonies within 24 hours. Growth is more luxuriant on semisolid agar. Uniform turbidity is produced in broth; and when the test tube is shaken, the accumulated deposit rises in the form of a small cloud. Growth is considerably stimulated by the addition of glucose or serum to the medium. On gelatin the bacteria produce a characteristic racemose growth. Two types of colonies are formed on gelatin plates, (1) those resembling a hazy spot and (2) fimbriate colonies. The organisms dissociate into S- and R-forms on agar.

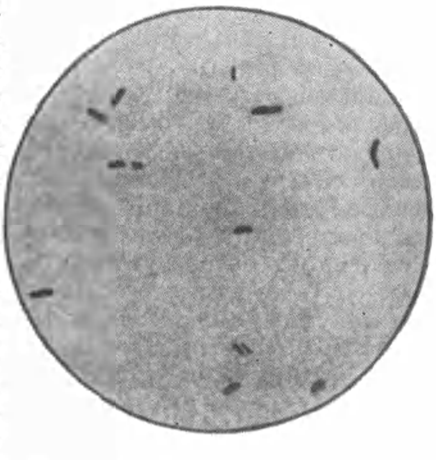


Fig. 116. *Erysipelothrix insidiosa*

Fermentative properties. The causative agent of erysipeloid does not liquefy gelatin and does not coagulate milk. Lactose, glucose, levulose, and galactose are fermented with acid production. The biochemical properties of *E. insidiosa* are extremely variable.

Toxin production. The organism does not produce an exotoxin. Diseases are mainly septic or allergic in character.

Antigenic structure and classification. *E. insidiosa* contains two antigens: a thermolabile group antigen and a thermostable specific antigen. Some authors differentiate two types of the organism, A and B, type B being less virulent than type A. Immunization of animals with type B results in the production of antibodies against both types. Type A is more specific. It causes the elaboration of type-specific antibodies.

Resistance. *E. insidiosa* are relatively resistant. They remain viable in soil throughout the winter, and in foodstuffs, including salted and smoked foods, for several months. They withstand 80° C for

30-45 minutes but are extremely sensitive to ultraviolet rays and penicillin.

Pathogenicity for animals. The organisms are pathogenic for swine, particularly the young, and produce swine erysipelas. In mice they are responsible for septicaemia. There is evidence of erysipelas occurring among lambs, chickens, ducks, turkeys, pigeons, sparrows, and other birds. Guinea pigs and rabbits are susceptible to erysipeloid.

Pathogenesis and disease in man. The sources of infection are affected swine, mice, and fish, decaying animal cadavers, foodstuffs, soil, water, etc., contaminated with the pathogen. *E. insidiosa* is widespread in nature. Carrier incidence among healthy swine reaches 50 per cent.

The disease prevails among people who come into contact with contaminated pork, cattle meat, and fish. Great epidemiological significance is attributed to rats and mice which are capable of contracting the disease and infecting foodstuffs, water, and various objects. In erysipeloid the endotoxins liberated by the causative organisms bring about disturbances of the vascular tone and increased permeability of the capillary walls. A varicose thickening of the nerve fibres occurs. Predisposing factors such as the cooling of hands and long-term sensitization of the skin by heterogenic substances play a definite role in the pathogenesis of erysipeloid. Recovery from erysipeloid is usually possible. Recovery is rapid after injections of anti-erysipeloid serum and penicillin.

Laboratory diagnosis. Smears of discharge obtained from the site of inflammation are examined under the microscope. The pure culture is isolated and its pathogenicity tested by subcutaneous injection into mice and inoculation into the pectoral muscle of pigeons. The infected animals die in 3-5 days. The agglutination reaction is employed for diagnosis of swine erysipelas.

Treatment. Immune serum and antibiotics (penicillin, etc.) are used.

Prophylaxis. Precautionary measures consist of early recognition of affected swine, systematic extermination of rats, cleaning of the land, storage and transportation of meat and fish products with observance of sanitary rules. Sanitary protection of rivers, lakes, and seashores and observance of personal hygiene when working with animals suspected of being affected with swine erysipelas are of great significance in the prevention of erysipeloid.

PATHOGENIC MYCOBACTERIA

The genus *Mycobacterium*, family *Mycobacteriaceae*, order *Actinomycetales*, includes bacteria which are characterized by their ability to branch and by their acid-, alcohol-, and alkali-fast properties.

The genus *Mycobacterium* includes organisms responsible for tuberculosis and leprosy, and a number of acid-fast saprophytes which occur in the body of poikilotherms, on various cereals, in soil, manure, milk, butter, etc.

CAUSATIVE AGENT OF TUBERCULOSIS

The organism responsible for tuberculosis in man (*Mycobacterium tuberculosis*) was discovered in 1882 by R. Koch. He also studied problems concerning the pathogenesis of tuberculosis and immunity produced by the disease. A. Calmette's and Ch. Guérin's discovery in 1919 of the live vaccine against tuberculosis was a very important event since it permits widespread practice of specific preventive vaccination. The introduction of streptomycin, phthivazide, isoniazid, PAS, and other drugs has supplied modern medicine with powerful means of tuberculosis control.

Morphology. *Myco. tuberculosis* is a slender, straight or slightly curved rod, 0.5-4μ in length and 0.3μ in breadth. It may have small terminal swellings. The organisms are nonmotile, Gram-positive, pleomorphous, and do not form spores or capsules. They stain poorly by the ordinary methods but are stained well by the Ziehl-Neelsen method (Fig. 117, *4*).

Rod-like, thread-like, branching, granular (Fig. 118), coccoid, and filterable forms have been observed.

E. Metchnikoff and V. Kedrovsky observed certain forms in cultures, which were similar to actinomycetes.

A. Fontès and others have put forward evidence of the existence of filterable forms of *Myco. tuberculosis*. On being injected into guinea

pigs, they become acid-fast and may be seen under the light microscope. Occurrence of nonbacillary G-forms has also been ascertained, the majority of them occurring under unfavourable conditions.

Electron microscopy has revealed the presence of granules and vacuoles located terminally in the cells of mycobacteria. The cytoplasm of young cultures is homogeneous, while that of old cultures is granular. *Myco. tuberculosis* is acid-fast due to the fact that it contains mycolic acid and lipids.

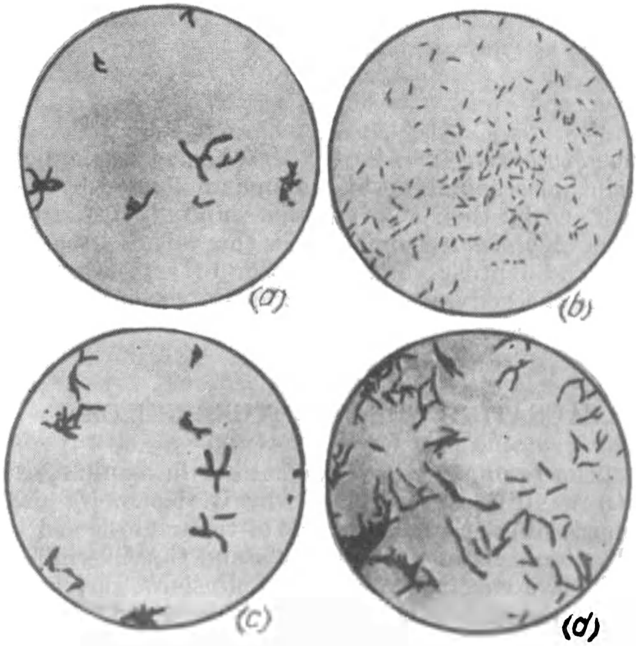


Fig. 118. *Mycobacterium tuberculosis*

a and *c*—branching forms; *b*—rod-like forms; *d*—granular forms

The lipids of *Myco. tuberculosis* consist of three fractions: (1) phosphatide which is soluble in ether; (2) fat which is soluble in ether and acetone; (3) wax which is soluble in chloroform and ether.

Nonacid-fast granular forms, which readily stain violet by Gram's method and known as Much's granules, and acid-fast Spengler's fragments of *Myco. tuberculosis* also occur.

Cultivation. The organisms grow on selective media, e.g., coagulated serum, glycerin agar, glycerin potato, glycerin broth and egg media (Petroff's, Petragnani's, Dorset's, Loewenstein's, Lubenau's,

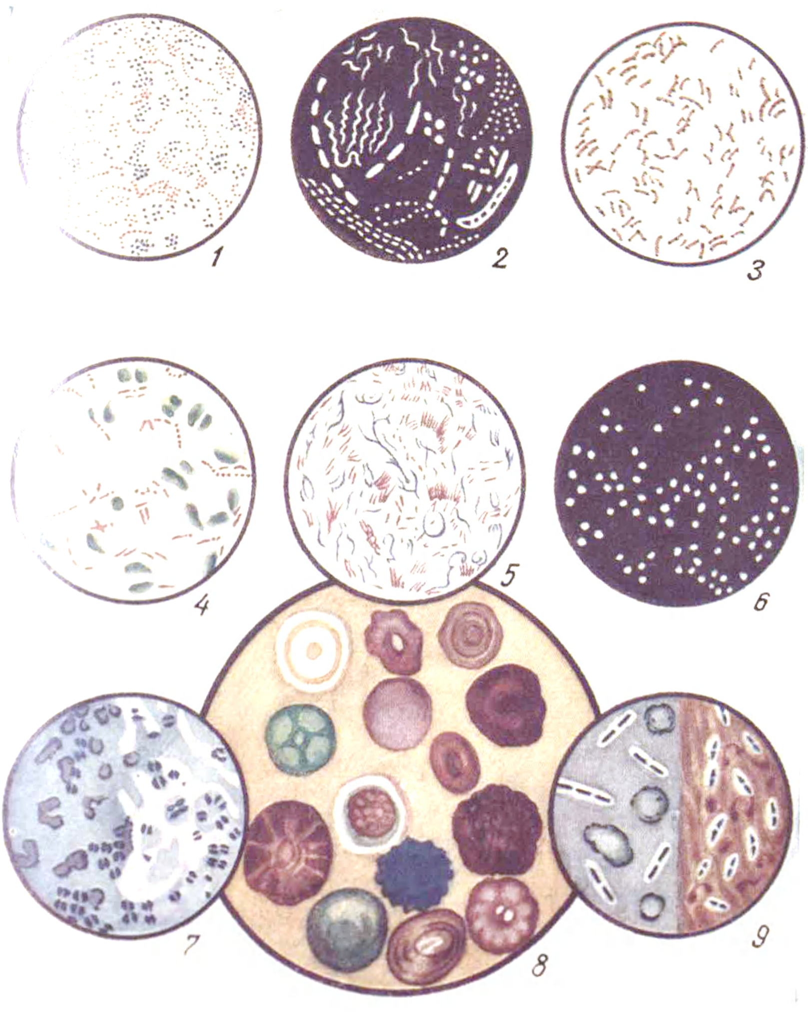


Fig. 117.

1—*Staphylococci* and *Brucella* organisms stained after Gram; *2*—organisms inhabiting the oral cavity. India-ink stained preparation; *3*—*Corynebacterium diphtheriae* stained after Neisser; *4*—*Mycobacterium tuberculosis* stained after Ziehl-Neelsen; *5*—*Mycobacterium leprae* stained after Ziehl-Neelsen; *6*—colonies of photobacteria photographed in their own light; *7*—gonococci stained with methylene blue; *8*—colonies of pigmented bacteria; *9*—*Bac. anthracis* and pneumococci with capsules (negative staining)

Vinogradov's, etc.). They may be cultured on Soton's synthetic medium which contains asparagine, glycerin, iron citrate, potassium phosphate, and other substances.

Certain levels of vitamins (biotin, nicotinic acid, riboflavin, etc.) are necessary for the growth of *Myco. tuberculosis*. Scarcely visible growth appears 8-10 days after inoculation on glycerin (2-3 per cent) agar, but in 2-3 weeks a dry cream-coloured pellicle is produced. The best and quickest (on the sixth-eighth day) growth is obtained on Petroff's egg medium which consists of egg yolk, meat extract, agar, glycerin, and gentian violet.

On glycerin (4-5 per cent) meat-peptone broth the organisms produce a thin delicate film in 10-15 days, which thickens gradually, becomes brittle, wrinkled, and yellow; the broth remains clear (Fig. 119).

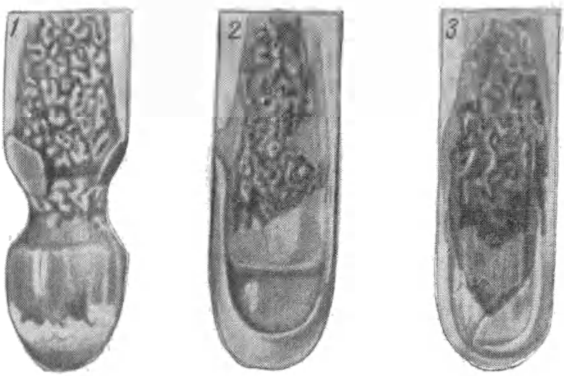


Fig. 119. Growth of *Mycobacterium tuberculosis*

1—on glycerol-potato agar; *2*—on solid egg medium; *3*—on solid egg medium (with an orange pigment)

Myco. tuberculosis can be successfully cultivated by Pryce's microculture method or Shkolnikova's deep method in citrated rabbit or sheep blood. Growth becomes visible in 3-6 days.

Synthetic and semisynthetic media are employed for cultivating *Myco. tuberculosis* in special laboratories.

The organisms are aerobic, and their optimal growth temperature is 37° C. They do not grow below 24 and above 42° C. The reaction of the medium is almost neutral (pH 6.8-7.2), but growth is possible at pH ranging from 6.0 to 8.0.

Myco. tuberculosis dissociate from typical R-forms to the atypical S-forms. Some strains produce a yellow pigment in old cultures.

Fermentative properties. The organisms have been found to contain proteolytic enzymes which break down proteins in alkaline and

acid medium. They also contain dehydrogenases which ferment amino acids, alcohols, glycerin, and numerous carbohydrates. *Myco. tuberculosis* is capable of causing reduction (they reduce salts of telluric acid, potassium tellurite, and break down olive and castor oils, etc.). The organisms produce lecithinase, glycerophosphatase, and urease which ferment lecithin, phosphatides, and urea.

Toxin production. *Myco. tuberculosis* does not produce an exotoxin. It contains toxic substances which are liberated when the cell decomposes.

In 1890 R. Koch isolated a substance known as tuberculin from the tubercle bacillus. There are several tuberculin preparations. The Old Koch's tuberculin is a 5-6-week-old glycerin broth culture sterilized for 30 minutes by a continuous current of steam (100°C), evaporated at 70°C to one tenth of the initial volume, and filtered through a porcelain filter. The New Koch's tuberculin consists of desiccated *Myco. tuberculosis* which are triturated in 50 per cent glycerin to a homogeneous mass. A tuberculin has been derived from the bovine variety of *Myco. tuberculosis*, which contains protein substances, fatty acids, lipids, neutral fats, and crystalline alcohol. There is also a tuberculin free of waste substances and designated PPD (purified protein derivative) or PT (purificatum tuberculinum).

Tuberculin is toxic for guinea pigs which are affected with tuberculosis (injection of 0.1 ml of the standard preparation is fatal for 50 per cent of experimental animals). Small doses of tuberculin produce no changes in healthy guinea pigs.

The chemical composition of the toxic substances contained in *Myco. tuberculosis* has not yet been ascertained. It is known that the toxin of the tubercle mycobacteria is composed of proteins (albumins and nucleoproteins). Phosphatides have been isolated from the virulent types of the organism and are capable of producing characteristic lesions in rabbits. Phthioic acid is the most active.

Extremely toxic substances have been extracted from *Myco. tuberculosis* after boiling in vaseline oil. They are fatal to guinea pigs in doses of one-thousandth of a milligram.

Virulent mycobacteria differ from the nonvirulent organisms in that they contain a great number of lipo-polysaccharide components.

Antigenic structure. On the basis of agglutination and complement-fixation reactions a number of types of mycobacteria have been distinguished: mammalian (human, bovine, and rodent), avian, poikilotherm, and saprophytic. The human type does not differ serologically from the bovine or murine types. Mycobacterial antigens produce agglutinins, opsonins, precipitins, and complement-fixing bodies in low titres. Tuberculin is considered to be a peculiar antigen (hapten). Some authors regard it as a high molecular compound, others as a lipid, and still others as a low molecular compound. However, it has not been definitely ascertained which antigen is re-

sponsible for the production of immunity. Only a high molecular tuberculin may be considered to be a full-value antigen capable of stimulating the production of corresponding antibodies.

Myco. tuberculosis and tuberculin possess allergenic properties and produce local, focal, and generalized reactions in the body infected with tuberculosis.

According to data supplied by a number of investigators, the *Myco. tuberculosis* antigen contains proteins, lipids, and particularly large amounts of phosphatides and lipo-polysaccharides. Experiments on animals have proved that the lipo-polysaccharide-protein complexes protect the body from infection with *Myco. tuberculosis.*

Tuberculin is widely used for allergic tests, e.g., the Pirquet reaction and Mantoux test in human beings and Koch's and Calmette's reactions in animals. These tests are employed for the determination of infection with *Myco. tuberculosis.*

Classification of mycobacteria which are pathogenic for human beings, cattle, rodents, and birds is given in Table 30. There are also strains of *Myco. tuberculosis* which affect poikilotherms and acid-fast saprophytes.

Besides the main types, there are atypical acid-fast mycobacteria (paratuberculosis) assumed by some investigators to be mutants or variants of tubercle mycobacteria which form in the patient's body under the influence of antibacterial therapy and defense mechanisms of the infected macroorganism. They are subdivided into three groups: (1) photochromogens which are mainly isolated from human beings during the disease; these organisms produce a lemon-yellow pigment in the culture when exposed to light, produce no pigment on culture in the dark, are polymorphous, and of low virulence for guinea pigs and highly virulent for mice on intravenous inoculation; (2) nonphotochromogens which are white, greyish, or yellowish in colour (pigment forming without the influence of light), resistant to PAS, nonvirulent for guinea pigs and rabbits but pathogenic for mice and hamsters; (3) scotochromogens which develop a bright-orange pigmentation when cultured in the dark and are more resistant to antibacterial preparations than the typical tubercle mycobacteria.

Resistance. Tubercle bacilli are more resistant to external effects as compared to other nonsporeforming bacteria as a result of their high lipid content (25-40 per cent).

The organisms survive in the flowing water for over a year, in soil and manure up to 6 months, on the pages of books over a period of 3 months, in dried sputum for 2 months, in distilled water for several weeks, and in gastric juice for 6 hours. They are easily rendered harmless at temperatures ranging from 100 to 120°C. The organisms are sensitive to exposure to sunlight.

Pathogenicity for animals. Tuberculosis is an infection which is widespread among cattle, chickens, turkeys, etc. Swine, sheep and goats contract the disease less frequently.

Table 30

General Characteristics of Pathogenic Mycobacteria

Name	Morphology	Type of colonies	Rate of growth, days	Optimal growth temperature
Mycobacterium tuberculosis	Thin, granular, and slightly curved	Rough, flat. R-forms are pathogenic	15-20	37-37.5°C
Mycobacterium bovis	Thick, short	Smooth, hemispherical, small. S-(or R-) forms are pathogenic	25-30	37-37.5°C
Mycobacterium microti	Thin, pleomorphous	Rough. R-forms are pathogenic	30-40	37°C
Mycobacterium avium	Thin, granular	Smooth, hemispherical. S-forms are pathogenic	10-20	41-42°C (45°C)
Mycobacterium paratuberculosis	Thick, short, pleomorphous, segmented	Colourless, yellowish-orange, wrinkled, dry. R-forms are pathogenic	40-60	38.5°C
Mycobacterium leprae	Thin, granular, straight	—	—	—
Mycobacterium lepraemurium	Thin, long	Do not grow on artificial media		

Cattle, sheep and goats are quite resistant to the human type of tubercle mycobacteria. Guinea pigs are highly susceptible to the human type, and their infection results in a generalized pathological condition and death. Infection of rabbits produces chronic tuberculosis.

The bovine type of the organism is pathogenic for many species of domestic mammals (cows, sheep, goats, pigs, horses, cats, and dogs) and wild animals. Infected rabbits and guinea pigs contract acute tuberculosis, the condition always terminating in death.

Cattle and, less frequently, sheep and goats contract paratuberculosis (Johne's disease, a chronic specific hypertrophic enteritis) which is caused by *Mycobacterium paratuberculosis*. The disease is characterized by a prolonged latent period and a chronic course with manifestations of intestinal dyspepsia and diarrhoea. The animals die as a result of exhaustion. Paratuberculosis epizootics sometimes occur.

The avian type of tubercle mycobacteria produces infection in chickens, turkeys, guinea fowls, peacocks, pheasants, pigeons, and waterfowl in natural conditions. Domestic animals (horses, pigs, goats, and less frequently cattle) may naturally acquire the disease by infection with the avian type organisms. Human beings may also be infected in some cases.

Among laboratory animals rabbits are highly susceptible to the avian type of tuberculosis, small doses of the organism causing generalized tuberculosis. Guinea pigs are relatively resistant and subcu-

taneous injections of the culture affect the lymph nodes, which is accompanied by the development of caseous foci.

The murine type of *Myco. tuberculosis* is extremely pathogenic for field mice. Experimental inoculation of rabbits and guinea pigs with this type of mycobacteria produces chronic tuberculosis.

Pathogenesis and disease in man. It has been shown that tuberculosis in human beings is caused by two types of mycobacteria—the human type (*Mycobacterium tuberculosis*) and the bovine type (*Mycobacterium bovis*). The human type is responsible for 90 per cent of the cases and the bovine type for the remaining 10 per cent. The avian type of *Myco. tuberculosis* may also be the cause of human tuberculosis in some cases (on ingestion of insufficiently cooked chicken meat and eggs contaminated with *Myco. avium*).

Infection with tuberculosis takes place through the respiratory tract by the droplets and dust, and, sometimes, per os through contaminated foodstuffs, and through the skin and mucous membranes. Intrauterine infection via the placenta may also occur.

With air-borne infection, the primary infectious centre develops in the lungs, but if infection takes place through the alimentary tract, the primary focus is in the mesenteric lymph nodes. When body resistance is low and conditions of work and life are unfavourable, the organisms may leave the site of primary localization and spread throughout the body, causing a generalized infection. At present, there is a point of view which maintains that localization of the infectious focus in the lungs is preceded by a lympho-haematogenic dispersion of *Myco. tuberculosis* throughout the body.

The development of the primary tuberculous foci takes a benign course if the conditions of life are favourable and there are no aggravating factors present. This stage usually terminates with resorption and healing of the caseous foci which become calcified and enclosed in a dense connective-tissue capsule. However, such result is not accompanied by the body becoming completely freed from the causative agents. About 70 per cent of people who are under 20 years of age are infected with *Myco. tuberculosis* but no disease is produced in them.

The organisms survive in the lymph nodes and other tissues and organs of the primary focus for many years and sometimes even for life. People infected in such a way acquire, on the one hand, relative immunity and, on the other hand, a potentially latent form of tuberculosis which may become active under the influence of a number of infectious diseases and psychic and physical traumas.

In some cases primary tuberculosis can be quite severe in noninfected and nonimmunized people, particularly if they were infected by massive doses as a result of contact with patients who discharge virulent mycobacteria.

Incidence of re-infection with tuberculosis increases 3-5-fold among individuals exposed to exogenous superinfection and the

resulting condition is more severe than aggravation of primary tuberculosis. It involves the development of new foci in the lymphatic system, increased sensitization, and accumulation of irritations as a result of the body being affected by pathogenic mycobacteria which are extreme irritants.

Tuberculosis is characterized by a variety of clinical forms, anatomical changes, compensational processes, and results. The infection may become generalized and involve the urogenital organs, bones, joints, meninges, skin, and eyes.

Immunity. Man is naturally resistant to tuberculosis, this property being hereditary. On the basis of the allergic reaction, X-ray examination, and patho-anatomical changes it has been shown that in a great number of cases infection does not result in disease.

There is a characteristic immunity produced by tuberculosis. Inoculation of *Myco. tuberculosis* into healthy guinea pigs causes no visible changes during the first days after infection. But a compact tubercle which undergoes ulceration is formed in 10-14 days. The lymph nodes become enlarged and hard, a generalized process develops, and the animal dies.

When tuberculous animals are inoculated with *Myco. tuberculosis*, an ulcer is formed at the site of injection. This ulcer shortly heals and no involvement of the lymph nodes or generalization of infection takes place. These facts were established by Koch and advanced the knowledge on a number of problems concerning pathogenesis and immunity in tuberculosis. Particular importance was attributed to nonsterile (infectious) immunity which has been widely reproduced artificially (by BCG vaccination). It is understood that immunity to tuberculosis is usually nonsterile. However, as in brucellosis, the phase of nonsterile immunity in tuberculosis is followed by the phase of sterile immunity.

Agglutinins, precipitins, opsonins, lysins, and complement-fixing antibodies are found to be present in the sera of tuberculosis patients. The presence of these substances, however, provides no evidence of the intensity of the immunity. Likewise, insusceptibility cannot be determined by the phagocytic reaction since phagocytosis in tuberculosis is frequently incomplete. Body reactivity and specific productive inflammation play the main role in production of immunity. This inflammation renders the *Myco. tuberculosis* harmless by formation of granulomas which consist of epithelioid cells surrounded by a zone of lymphoid and giant Langhans' cells.

Interference of *Myco. tuberculosis* with BCG strains and other nonvirulent mycobacteria which are capable of blocking tissue and organ cells sensitive to virulent tuberculous mycobacteria plays a definite role in the complex of defense mechanisms of the body.

A new component which affects *Myco. tuberculosis* has been found to be present in human blood. Individuals devoid of this component are more susceptible to tuberculosis.

Among the defense factors phages should be mentioned. They affect both virulent and avirulent *Myco. tuberculosis* strains. The discovery of phages is of certain practical importance. They may be used in diagnosis of tuberculosis and, probably, in the treatment of the disease.

Many tissues are capable of producing enzymes which break down mycobacteria. Such properties are characteristic of enzymes of the nuclease group.

The barrier function of tissues and organs which stops the organisms and prevents their dispersal throughout the body is of essential importance in body resistance to tuberculosis. Antituberculous antibacterial agents which have been found in the blood, muscles, skin, thyroid gland, pancreas, spleen, and kidneys are also of great significance. The role of tuberculous allergy in immunity has not been ascertained, although various points of view on this subject have been expressed (see the section "Relation of Allergy to Immunity"). The majority of phthisiotherapists hold that there is no correlation between allergy and immunity in tuberculosis.

Laboratory diagnosis. 1. *Microscopy of smears from sputum, pus, spinal or pleural fluid, urine, faeces, lymph nodes, etc., stained by the Ziehl-Neelsen method* (see Fig. 117, *4*).

For concentration of the organisms, the sputum is subjected to enrichment methods:

(a) homogenization (an equal volume of 1 per cent NaOH solution is added to the sputum, the flask is tightly stoppered and shaken for 5-15 minutes until the sputum is dissolved completely; after centrifugation, the precipitate is neutralized by one or two drops of a 10 per cent hydrochloric acid solution and smears are prepared);

(b) flotation (the homogenized sputum is transferred into a flask which has a rubber stopper and heated in a water bath at 55°C for 30 minutes, after which it is diluted with distilled water, and 1 or 2 ml of xylol, benzine or gasoline are added; the mixture is shaken for 10 minutes and after it has been left to settle for 30 minutes, smears are made from the resulting cream-like layer).

There are other methods of sputum preparation which facilitate the demonstration of mycobacteria.

Good results are obtained by employing luminescent microscopy with auramine and examining the specimens under the phase-contrast microscope.

2. *Isolation of the pure culture.* The prepared sputum, pus, suspensions of parenchymatous tissues, and other material are inoculated into nutrient media.

Pryce's microculture method is the most effective. The material under test is spread thickly on a slide, dried, and treated with sulphuric acid which is then washed off with a sterile sodium chloride solution. The preparations are then put into flasks containing citrated blood and placed into a thermostat for a period of 2-3 days, or a

maximum of 7-10 days. The preparations may be stained after 48 hours' incubation. Virulent mycobacteria produce convoluted strands in the microcultures, while the nonvirulent strains form amorphous clusters.

The virulent and nonvirulent *Myco. tuberculosis* strains are differentiated by their growth on butyrate albumin agar (Middlebrook-Dubos test). The virulent strains grow in the form of plaits (Fig. 120), and the nonvirulent strains form irregular clusters. The above mentioned authors suggested the differentiation of the virulent and nonvirulent strains by staining the smears with neutral red which has an affinity for virulent mycobacteria and stains them purple-pink (nonvirulent strains are stained yellow).

Fig. 120. Growth of virulent *Mycobacterium tuberculosis* strains on butyrate albumin agar

3. *Biological method.* Inoculation of guinea pigs produces an infiltrate at the site of injection of the material, lymph node enlargement, and generalized tuberculosis. The animals die 1-$1^1/_2$ months after inoculation. Post-mortem examination reveals the presence of numerous tubercles in the internal organs. Specimens are obtained from lymph nodes by puncture 5-10 days after inoculation and examined for the presence of tubercle bacilli. The tuberculin test is carried out 3-4 weeks after infection.

4. *Complement-fixation reaction* (positive in 80 per cent of cases with chronic pulmonary tuberculosis, in 20-25 per cent of patients with skin tuberculosis, and in 5-10 per cent of healthy people).

5. *Indirect haemagglutination reaction* (Middlebrook-Dubos test). Sheep erythrocytes, on which polysaccharides of *Myco. tuberculosis* or tuberculin are adsorbed, are agglutinated in serum of tuberculosis patients.

6. *Tuberculin (allergic) tests* (Pirquet's reaction and the Mantoux test) are used for detecting infection of children with *Myco. tuberculosis* and for diagnosis of tuberculosis.

Treatment is accomplished with antibacterial preparations. They include derivatives of isonicotinic acid hydrazide (tubazide, phthivazide, methazide, etc.), streptomycin, and PAS—preparations of the first series. Preparations of the second series (cycloserine, ethox-

ide, ethyonamide, pyrazinamide, capryomycin, etc.) are used with an aim to enhance the therapeutic effect. Surgical and climatic (health resort) treatment is also beneficial in certain cases. The complex of therapeutic measures for body desensitization includes the use of tuberculin and elpimed. Elpimed is a substance derived from horse serum and contains unsaturated fatty acids. It restores body reactivity. Combined treatment with preparations of the first and second series is recommended in chronic forms of tuberculosis. Good effect is rendered, in particular, by a combination of cycloserine and elpimed.

At present, in certain cases patients are given prednison together with chemotherapeutic agents and antibiotics. Tuberculin therapy is applied in insipient forms of primary tuberculosis.

Prophylaxis is insured by early diagnosis, timely detection of patients with atypical forms of the disease, routine check up of patients and recovered patients, disinfection of milk and meat derived from sick animals, and other measures.

Active immunization of human beings is of great importance in the control of tuberculosis. It lowers significantly the incidence of the disease and the death rate, gives protection against the development of severe cases, and lowers the body sensitivity to the effect of tubercle mycobacteria and to the products of their disintegration. Active immunity makes the body capable of fixing and rendering harmless the causative agent, stimulates biochemical activity of tissues and intensifies the production of antibacterial substances. Immunization produces a certain type of infectious immunity.

Intracutaneous immunization and revaccination have been carried out in the USSR since 1962. For these requirements a special dry BCG vaccine is produced. Newborn infants are given a 0.01 mg dose, children under 7 years of age are given 0.02 mg of the preparation, and schoolchildren, adolescents, and adults are given a 0.05 mg dose. Revaccination is carried out at the age of 7, 11-12, 14-15, 17-18, 22-23, and 27-30 years.

Postvaccinal immunity is produced within 3 or 4 weeks and remains for $1\text{-}1^1/_2$ years. It is associated with a change in body reactivity, the body becoming capable of rendering harmless the tubercle bacilli.

Peroral antituberculosis vaccination with large doses (de Assisa's method) is widely used in Brazil and other countries. The vaccine is given 6 times in 100 mg doses.

Vaccine strains obtained from cultures isolated from field mice are at present being tested. The murine type (OVS strain) of *Myco. tuberculosis* is completely harmless to many animals but, nevertheless, it possesses immunogenic properties and produces immunity against human and bovine tuberculosis.

Living conditions play an important part in the incidence of tuberculosis. Deterioration of the conditions increases the incidence

of the disease and death rate (wars, famine, unemployment, economical crises, and other disasters). More than 14 million people suffer from tuberculosis in Japan. There are more than 1 million patients in Turkey, about 60,000 of which die every year. The mortality rate is quite high in India and Africa. Favourable conditions have been created in the USSR for successful control of tuberculosis and complete eradication of the disease in the next 10-11 years.

CAUSATIVE AGENT OF LEPROSY

The organism responsible for leprosy, *Mycobacterium leprae*, was discovered in 1874 by the Norwegian investigator A. Hansen. In 1901 V. Kedrowsky reported nonacid-fast forms of the organism and described their branching.

Morphology. *Myco. leprae* have many properties in common with the tubercle bacilli. They are straight or slightly curved bacilli, and club-shaped swellings and granular forms sometimes occur. The organisms are 1-8 μ in length and 0.3-0.5 μ in breadth. They usually occur in groups resembling packets of cigars (see Fig. 117, *5*) or clusters. They decolour more easily than *Myco. tuberculosis*. *Myco. leprae* is nonmotile, produces neither spores nor capsules, and is Gram-positive.

The organisms are pleomorphous. Among the more typical forms long, short, and thin cells as well as larger cells which are swollen, curved, branched, segmented, or degenerate (splitting up into granules) may occur.

Myco. leprae are similar to *Myco. tuberculosis* in chemical composition. Their lipid content ranges from 9.7 to 18.6 per cent. Besides mycolic acid, they contain laeprosinic oxy acid, free fatty acids, wax (leprozine), alcohols, and polysaccharides.

Cultivation. Attempts to cultivate *Myco. leprae* on nutrient media employed for growth of *Myco. tuberculosis* have been unsuccessful. The *Myco. leprae* are cultivated on fluid egg media which contain spermatic lysates of animals and fish. A fragile pellicle, yellow pigment, and a granular precipitate are produced in the medium. However, the cultured forms of *Myco. leprae* differ significantly from the organisms which occur in tissues and are nonvirulent for animals and human beings.

Experiments in which pieces of leproma enclosed in colloidal sacs were introduced into the peritoneal cavity of animals demonstrated the existence of a great variety of leprosy mycobacteria (nonacid-fast, capsulated, granular, coccal, spore-like, thread-like, and rod-like) which resemble fungal mycelium.

Fermentative properties have been insufficiently studied. This research has been handicapped by failure to solve the problem of cultivation of *Myco. leprae* on nutrient media.

Toxin **production**. The organisms have not been shown to produce a toxin, but they probably produce allergic substances. As no cultures of the organism have yet been obtained and no susceptible animal in which the disease can be reproduced experimentally has been found, the study of this problem is a matter of difficulty.

Antigenic structure and classification have not been worked out.

Resistance. *Myco. leprae* are extremely resistant, and survive in human corpses for several years. Although the organisms retain their morphological and staining properties outside the human body for a long period of time, they quickly lose their viability.

Pathogenicity for animals. Leprosy-like diseases are known to occur among rats, buffaloes, and certain species of birds, but they differ essentially from human leprosy. *Myco. leprae* is pathogenic only for human beings.

Pathogenesis and disease in man. Leprosy was well known in Egypt 3,000-4,000 years B.C. In the Middle Ages and during the Crusades, leprosy spread as epidemics. This period was characterized by continuous wars which caused bad sanitary conditions. There were 2,000 lepra colonies in France in 1429.

Leprosy disappeared from Europe at the end of the seventeenth century. In France all lepra colonies were closed on August 24, 1693. A new increase in the disease incidence occurred from 1867, followed by a marked decline at the beginning of the twentieth century. However, disease prevalence is still high. According to data furnished by the World Health Organization, over 10-12 million leprosy patients are registered throughout the world.

The source of infection is a sick person. The causative agent is transmitted by the air-droplet route through the nasopharynx and injured skin. The infection may also be spread by various objects. However, intimate and prolonged contact between healthy individuals and leprosy patients is the main mode of infection.

After entering the body through the skin and mucous membranes, *Myco. leprae* organisms penetrate into the nerve endings, lymphatic and blood vessels, and disseminate gradually without causing any changes at the site of entry. In the presence of high body resistance, the majority of *Myco. leprae* perish. In some cases infection leads to the development of latent forms of leprosy. The duration of such latent forms depends on body resistance, and may persist for a lifetime and, as a rule, terminates in the death of the causative agent. The latent form may change to the active form with development of the disease, if living and working conditions become unfavourable. The incubation period may last for years, e.g., from a period of 3-5 to 20-35 years. The disease becomes chronic.

Three types of leprosy are distinguished on the basis of clinical manifestations: lepromatous, tuberculoid, and undifferentiated.

1. The lepromatous type is characterized by minimum body resistance to the presence, multiplication, and spread of the causative

agent. *Myco. leprae* are constantly present at the sites of the lesions. The lepromin test is negative.

2. The tuberculoid type is distinguished by high body resistance to the multiplication and spread of *Myco. leprae.* Either no organisms are found at the site of the lesion, or only a small number of them may be present during the reactive state. The allergic test is usually positive.

3. The undifferentiated type (nonspecific group) is characterized by varying body resistance, but tends to be resistant. Microscopic examination does not always reveal the presence of *Myco. leprae.* Allergic tests are negative or yield a slightly positive reaction.

Immunity. Little is known about immunity in connection with leprosy. Patients' blood contains complement-fixing substances. Phagocytosis does not play any significant role in leprosy. An allergic condition develops during the course of the disease. The mechanism of immunity in leprosy is similar to that in tuberculosis.

In individuals with high body resistance, the organisms are phagocytosed by histiocytes in which they are destroyed quite rapidly. In such cases leprosy assumes a benign tuberculoid type.

In individuals with low resistance, *Myco. leprae* multiply in great numbers even within the phagocytes (incomplete phagocytosis), and the organisms disseminate throughout the body. A severe lepromatous type of the disease develops in such individuals.

Resistance may vary from high to low in undifferentiated types of leprosy. Relatively benign lesions persist for years, but if body resistance lowers the disease assumes a lepromatous form with large numbers of mycobacteria present in the tissues and organs. The clinical picture changes to the tuberculoid type when immunity intensifies.

Immunity in leprosy is associated with the general condition of the host body. In the majority of cases the disease occurs among the poor who have a low standard of culture. Children are most susceptible to the disease. In 5 per cent of cases the disease is acquired through contact with sick parents.

Laboratory diagnosis. Specimens for examination are obtained from nasal mucosa scrapings (on both sides), skin lepromas, sputum, and ulcer excretions. Blood is examined during the fever period. Microscopic examination is the principal method of leprosy diagnosis. Smears are stained with the Ziehl-Neelsen stain (see Fig. 117, *5*).

Biopsy of leprotic lesions and puncture of lymph nodes are employed in some cases. *Myco. leprae* can be seen as clusters resembling packets of cigars; in preparations from nasal mucus they have the appearance of red balls.

Leprosy is differentiated from tuberculosis by inoculating guinea pigs with a suspension of the pathological material in an 0.85 per cent solution of common salt. If tubercle bacilli are present, the ani-

mals contract the disease and die. Guinea pigs are insusceptible to *Myco. leprae*.

The allergic Mitsuda test is considered positive when an erythema and a small papule (early reaction) are produced at the site of an 0.1 ml lepromin (a suspension prepared from a leproma after trituration and prolonged boiling) injection in 48-72 hours; this reaction either disappears completely at the end of the first week or changes to the late reaction. The latter is manifested by a nodule which appears at the site of injection in 10-14 days, and grows to a diameter of 1-2 cm with necrosis in the centre. This test is of no diagnostic value and is used to distinguish the clinical type of leprosy.

The complement-fixation reaction and the Middlebrook-Dubos haemagglutination test are employed for leprosy diagnosis.

Treatment. Leprosy is treated with sulphone drugs, diaminodiphenylsulphone and its derivatives (sulphetrone, promin, diazone, and promacetin). Carbonylid (Su 1906) is less toxic. In addition to this, conteben, desensitizing agents, and corticosteroid preparations (cortisone, prednisolone, etc.) are employed. Streptomycin and dehydrostreptomycin combined with PAS and isoniazid, and tybon, phthivazide, and biostimulators yield good effects.

For a long period of time, leprosy patients were treated with chaulmoogra oil which was given per os. At present it is administered intramuscularly or intracutaneously. Chaulmoogra preparations promote the resolution of lesions and, sometimes, eliminate the visible leprosy manifestations. However, they give no protection from relapses and have no specific effect.

Prophylaxis. Leprosy patients which discharge the organisms are isolated in lepra colonies till clinical recovery. Patients who do not discharge leprosy organisms receive out-patient treatment. Routine epidemiologic control of endemic foci is carried out. If there is a leprosy patient in a family, all other members are subjected to a special medical examination at least once a year. Children born of mothers with leprosy should be taken away from them and fed artificially. Healthy children of leprosy parents are placed in children's homes or are looked after by relatives and are examined at least twice a year.

In the USSR, leprosy has become a sporadic disease. Only isolated cases are registered in some regions of the country.

PATHOGENIC ACTINOMYCES

The organisms responsible for actinomycosis were discovered in 1877 by C. Harz and isolated in a pure culture in 1891 by E. Bostroem. Actinomyces belong to the class *Schizomycetes*, order *Actinomycetales*, family *Actinomycetaceae*.

Morphology. In relation to their properties, actinomyces are intermediate between bacteria and fungi. In contrast to bacteria, they have a distinct nonseptate mycelium with aerial sporangia. The hyphae (mycelium filaments) are 100-600 μ long and 0.5-1.2 μ thick. The organisms stain well with all aniline dyes and are Gram-positive. The hyphae of some actinomyces are encapsulated.

Cultivation. Actinomyces grow readily at 25-30°C (the optimal growth temperature being 37°C) on ordinary media with the pH adjusted to 4.4-9.0 and under aerobic conditions. Anaerobic species also occur. The organisms are acid-fast, and in fresh cultures they form granules terminally. On solid media some species produce dense smooth colonies, while colonies of other species are raised, wart-like, crusty, velvety, fluffy, or farinaceous and adhere to the medium so that it is difficult to remove them with a loop. The colonies are either colourless or pigmented (blue, violet, red, yellow, orange, green, etc.).

On solid nutrient media actinomyces frequently produce aerial mycelium. Spores produced at the ends of the mycelium impart a definite colour to the colonies.

Fermentative properties. The pathogenic species comprise: (1) *Actinomyces bovis* which ferments proteins with hydrogen sulphide production, causes coagulation followed by peptonization of milk, hydrolyses starch, and ferments saccharose, maltose, and glucose, with acid production, and neither liquefies gelatin nor breaks down erythrocytes; (2) *Actinomyces israelii* which does not liquefy gelatin or produce haemolysis and which ferments mannitol, saccharose, lactose, maltose, and glucose, with acid formation; (3) *Actinomyces baudetti* which does not liquefy gelatin, does not coagulate milk, but ferments starch, saccharose, and glucose.

The problem of **toxin production** is a debatable point, some authors maintaining that pathogenic actinomyces contain endotoxins.

Resistance. The organisms are very resistant, withstanding a temperature of 60°C for 1 hour and remaining viable for a long period of time when dried. The spores are particularly resistant.

Pathogenicity for animals. Pathogenic actinomyces produce lesions in cattle and, less frequently, in swine and horses. Diseases become chronic and are manifested by inflamed centres and fistulae. It has now been ascertained that certain species of actinomyces may produce actinomycosis not only by themselves but also in association with pyogenic bacteria. The skin, tongue, lips, cheeks, and neck are most frequently involved. The bones and udders are sometimes affected.

Pathogenesis and disease in man. Cattle, sheep, goats, horses, pigs, dogs, rabbits, wild animals and certain environmental objects (soil, plants, air, and various grain scrap) may be sources of infection. Actinomycosis may result from endogenous infection when the causative agent penetrates from the digestive tract. The chewing of grain, various skin and mucosa injuries, and, particularly, carious teeth facilitate infection.

Actinomyces israelii occurs more frequently and is more widespread than other causative agents of actinomycosis.

Having penetrated the body, the causative agent spreads gradually along the strata in the connective tissue and between the muscles and is circulated in the blood and lymph.

The infectious process is accompanied by the formation of infiltrates, suppurative foci, and fistulae which open within or outside the body. The disease is characterized by chronic inflammation with subsequent development of suppurative processes. Hard, phlegmon-like infiltrates or granulomas form at the site of localization of the organisms. The skin becomes purple-blue, and the infiltrates soften, necrotize, and discharge pus which has an offensive odour. Granules (drusens), consisting of threads of actinomyces (Fig. 121), are present in the pus. Affection with pathogenic actinomyces is, as a rule, accompanied by secondary pyogenic infection. Actinomycosis is therefore considered to be a polybacterial disease at present.

Fig. 121. Drusen of pathogenic actinomyces

According to clinical manifestations, actinomycosis of the face and neck, pulmonary actinomy-

cosis, abdominal actinomycosis, actinomycosis of the internal organs, skin actinomycosis, skin and muscular actinomycosis, bone and muscular actinomycosis, actinomycosis of the nose, ears, larynx, pharynx, eyes, central nervous system, etc., can be differentiated.

Immunity. Actinomycosis does not leave immunity, and reinfections may occur. Agglutinins, precipitins, and complement-fixing antibodies are present in human and animal blood during the disease and after recovery. The presence of these substances, however, does not confer insusceptibility. Infectious and postinfectious immunity are accompanied by allergy.

Laboratory diagnosis. The following procedures are carried out:

(1) stained and unstained pus preparations are examined for the presence of drusens;

(2) pus is inoculated into sugar broth with the pH adjusted to 6.8, sugar agar, or Sabouraud's medium (100 ml of yeast water, 1 g of peptone, 4 g of maltose, and 2 g of agar are sterilized at 0.5 atm for 20 minutes) and grown under aerobic and anaerobic conditions at 35-37°C;

(3) complement-fixation reaction with patient's serum is carried out;

(4) allergic test (intracutaneous test with actinomyces extracts) is performed.

Treatment is accomplished with actinolysates, sulphadimezin, penicillin, streptomycin, chloromycetin, chlortetracycline, oxytetracycline, phthivazide, isoniazid, etc. These drugs are prescribed in combination with iodine, X-ray therapy and surgery.

Prophylaxis comprises observance of individual hygiene, removal of predisposing factors (skin and mucosa injuries in particular), care of the mouth and throat, teeth care, and hand washing.

The order *Nocardia* (E. Nocard, 1888) belongs to the family *Actinomycetaceae*. Some species of these organisms produce human diseases. The organisms occur in patients' pus and sputum in the form of branching filaments and rods. They are Gram-positive, acid-fast, and grow readily under anaerobic conditions on common media at 22-37°C. Actinomyces of this order are widely spread in nature. The pathogenic species are responsible for severe human diseases which resemble actinomycosis. The lungs are involved followed by pleuropneumonia or kidney metastases, skin abscesses, and the disease tends to spread throughout the body. The disease is characterized by the development of allergy. Laboratory diagnosis comprises the same methods as those employed for actinomycosis. Treatment is accomplished with sulphonamides, as antibiotics have no effect.

Human actinomycoses include Madura disease (Madura foot or maduromycosis) which is caused by *Nocardia madurae* (H. Vensent, 1894) and other actinomyces. It is a chronic foot infection, the

foot becoming swollen and deformed. At times, the process may be localized in the shank, hands, abdomen, etc. Following penetration of the organism, nodules are produced at site of entry, the skin becomes red-violet or brown, the nodules soften and break through the skin, forming fistulas which discharge pus. The pus contains white. yellow, or orange granules which are composed of interlaced mycelia, cell elements, and detritus. Patients are treated with penicillin in combination with sulphonamides. Chlortetracycline and oxytetracycline are also used.

CAUSATIVE AGENTS OF RELAPSING FEVER, FUSOSPIROCHAETOSIS, SYPHILIS, AND LEPTOSPIROSES

The general characteristics of the spirochaetes are given in the section "Morphology of Microorganisms" (p. 49-50). They possess no fermentative properties which provide information for laboratory diagnosis and produce no soluble toxins.

CAUSATIVE AGENTS OF RELAPSING FEVER

According to its vectors, relapsing fever is subdivided into two types: epidemic, transmitted by lice, and endemic, transmitted by ticks.

The causative agent of epidemic relapsing fever was discovered in 1868 by O. Obermeier.

Morphology. The organisms responsible for relapsing fever (*Borrelia recurrentis*) are thin spiral threads 8-16 μ long and 0.35-0.5 μ thick. They possess from four to twelve spirals, and their ends are pointed (Fig. 122). The organisms are motile, Gram-negative, and readily stain blue-violet by the Romanowsky-Giemsa method.

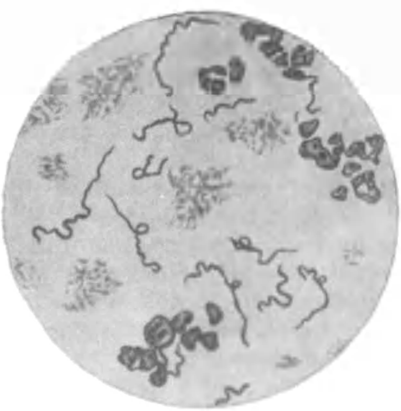


Fig. 122. Causative agent of relapsing fever

Cultivation. *Borrelia recurrentis* is cultivated under anaerobic conditions on nutrient media of pH 7.2-7.4 which contains ascitic fluid, serum, and pieces of tissues or organs. One or two drops of the patients' blood are inoculated into nutrient media, covered with oil, and incubated at 37°C. Cultures remain virulent for a long period of time (for several years).

Antigenic structure and classification. *Borrelia recurrentis* are nonfermentative organisms. There are no serological types.

Resistance. The organism survives in fluid media (in sealed glass tubes) at room temperature for as long as 14 days. It withstands deep freezing for 8 days and 0°C for 3 days. Exposure to 45-48°C kills the organism in 30 minutes.

Pathogenicity for animals. Animals do not naturally contract relapsing fever. Experimental infection of rats, white mice, and monkeys is a matter of great difficulty. Guinea pigs, rabbits, and white mice are insusceptible to the causative agent of European relapsing fever.

Pathogenesis and disease in man. In 1874 G. Minkh, and in 1881 E. Metchnikoff performed experiments on themselves and showed that the blood of relapsing fever patients is infectious. This fact was corroborated by experiments which were carried out in 1912-14 by Ch. Nicolle, E. Consey et al. The patient is the source of infection, and the body and head lice (*Pediculis vestimenti* and *Pediculis capitis*) are the vectors. The louse is infected by sucking the patient's blood and, 5-12 days later, it becomes capable of infecting human beings. The organisms gain entrance into the body when haemolymph of crushed lice is rubbed into the skin. Lice remain capable of conveying infection throughout their life (30-40 days for body lice, and 25-30 days for head lice). Transovarial transmission of the organisms does not occur in lice. Relapsing fever is prevalent in winter.

Borrelia recurrentis multiplies in the tissues of the reticuloendothelial system. At the end of the incubation period, a great number of the organisms invade the blood where some of them are destroyed by bactericidal substances. The elaborated endotoxin affects the central nervous system and causes toxicosis, fever, functional disturbances, and dystrophy in organs and tissues. The endotoxin affects the blood-vascular system with resulting spleen infarction and necroses in the spleen and liver.

Under the influence of antibodies, complex aggregations are formed as the result of the organisms being clumped with thrombocytes which are retained in the capillaries of the internal organs. The causative agents are destroyed by lysins and phagocytes. Those organisms which are located in deep tissues and in the central nervous system become resistant to their new environment. Their antigenic properties alter with the result that the antibodies produced after the first attack are no longer effective against the organisms. Multiplication of new varieties of *Borrelia recurrentis* brings about new attacks of the disease. These attacks vary from 3 to 5 in number and last until the host body renders harmless all the new varieties present.

The disease is characterized by high temperature (39-40°C), nausea, vomiting, and spleen enlargement. Fever lasts for 6-7 days

during the first attack, then the temperature falls and apyrexia or remission of 5-7 days' duration follows. Each new attack is of less duration than the previous one, while the period of apyrexia becomes longer.

Complications of a septic character occurred during epidemics of relapsing fever and were caused by *Salmonella hirschfeldii*. Abortion, encephalitis, parotitis, iritis, and iridocyclitis are among the complications.

Immunity in relapsing fever is characterized by the presence of antibodies (agglutinins, lysins, and thrombocytobarins which are responsible for the Rieckenberg-Brusin adhesion phenomenon).

Laboratory diagnosis comprises the following procedures:

1. Examination of thick films and smears of blood obtained during the febrile period. Specimens are stained by the Romanowsky-Giemsa or Burri's stains, by fuchsin or by silver impregnation; the organisms are examined for motility in the thick films by use of dark-field illumination; the concentration method is employed for detecting the organisms during the period of apyrexia (8-10 ml of patient's blood is coagulated and the serum is drawn off and centrifuged for 45-60 minutes at 3,000 revolutions per minute; after removing the supernatant, a thick film is made from the precipitate formed at the bottom of the tube and a drop of serum; the preparation is left to dry, fixed by Nikiforov's mixture, stained with Romanowsky-Giemsa stain, and examined under the microscope); this method is employed for retrospective diagnosis and for evaluation and control of specific therapy.

2. A serologic test may be carried out during the period of apyrexia. A drop of serum taken from a patient who has recovered from an attack of relapsing fever is mixed on a slide with a drop of patient's blood containing the causative agent. The slide is covered with a coverslip and placed in an incubation room. The *Borrelia* lose their motility and are destroyed in 30-60 minutes.

3. The Rieckenberg-Brusin test is performed as follows. The patient's serum is mixed with an equal volume of citrated plasma of a healthy guinea pig. One volume of the *Borrelia* culture is added to three volumes of this mixture, thoroughly stirred in a test tube, and placed in an incubation room at 37°C for 15 minutes. Then a drop is taken from the bottom of the test tube with a pipette, transferred to a slide, covered with a coverslip, and examined using dark-field microscopy and an immersion objective. In the presence of specific antibodies, the guinea pig thrombocytes are adsorbed on the surface of *Borrelia* cells and hinder their movement (the *Borrelia* are clumped with thrombocytes).

4. The biological method is employed for differentiating epidemic and endemic relapsing fever. A guinea pig is inoculated with 3-5 ml of patients' blood, and in the presence of endemic (tick) re-

lapsing fever, the animal becomes ill and *Borrelia* are easily revealed in the blood.

The presence of *Borrelia* in thick films or smears is a decisive factor in diagnosis.

Treatment is accomplished with penicillin, chlortetracycline, chloromycetin, and arsenic preparations (novarsenol [neoarsphenamine]).

Prophylaxis. Such disasters as wars, famine, and devastation may give rise to epidemics of relapsing fever. More than four million patients with relapsing fever and a death rate of 10-26 per cent were registered in Russia during the World War I. The disease incidence also increased during World War II, but the death rate did not exceed 1 per cent.

Improvement of living and cultural standards, the practice of antiepidemic precautions, timely recognition of the disease, hospitalization of patients, and medical control of sources of disease have led to complete elimination of relapsing fever in the USSR.

The organism responsible for tick-borne relapsing fever was discovered in the blood of patients in 1904 by P. Ross. Later, *Borrelia persica* (E. Dzhunkowsky, 1913), *Borrelia caucasica* (S. Kandelaki, 1928), *Borrelia latyschewii* (M. Sofiev, 1941), and other species of *Borrelia* were described. They all produce zoonotic infections.

Morphology. *Borrelia* responsible for tick-borne relapsing fever morphologically resemble the causative organisms of epidemic relapsing fever.

Cultivation. The organisms are grown on Geltser's medium which contains rabbit serum previously heated at 56-58°C, an equal volume of isotonic common salt solution, and pieces of coagulated albumin of the hen's egg.

Antigenic structure and classification. Several species of *Borrelia*, pathogenic for human beings and animals, are known. Three species have been described in the USSR. It has not been possible to differentiate them by their serological and morphological properties, the biological method yielding the best results.

Resistance. The resistance of the causative agents of tick-borne fever is similar to that of the *Borrelia* of louse-borne relapsing fever. However, the former are more resistant to salvarsan and its derivatives.

Pathogenicity for animals. In nature, the causative agents of tick-borne relapsing fever are parasitic within the body of wild rodents and insectivores from which they gain entrance into the body of *Ornithodorus* ticks. Among laboratory animals, the guinea pig is susceptible to *Borrelia* of tick-borne relapsing fever and insusceptible to *Borrelia* of louse-borne relapsing fever. White mice and rats are susceptible to both species of the organisms.

Pathogenesis and disease in man. Pathogenesis is similar to that of epidemic relapsing fever. Human tick-borne fever is endemic in character, prevailing in the warm months, mainly in the spring.

Burrows, cracks, caves, grottos, and dust of mudhouses and sheds serve as community habitats for ticks. It is here that the circulation of the causative agent from the wild mammals to the ticks and vice versa takes place.

Ticks are parasitic on rodents, which become the reservoir of infection. After entering the tick's gut, the organisms penetrate into the mouth cavity in 10-12 days and then spread throughout the body.

The penetration of *Borrelia* into the tick's oviduct and eggs provides the means for transovarial transmission of the causative agent. The tick remains infected for life, which may be for over 10 years. Human beings acquire the infection through tick bites, when a papula forms at the site of the bite (primary affect).

The disease is characterized by relapses of 1-2 days' duration. The relapses may be 5-7-9 and more in number. The duration of remissions varies from several hours to 6-8 days.

Immunity. The *Borrelia* of tick-borne and louse-borne relapsing fever differ in their immunological properties. Among the populations of endemic regions immunity is acquired from early childhood—a fact demonstrated by the detection of antibodies in the blood plasma of inhabitants. The disease occurs mainly among newcomers to such regions.

Laboratory diagnosis. The following methods are employed: (1) microscopic examination of the blood—in thick films and smears; (2) laboratory inoculation of guinea pigs (0.5-1 ml of blood is injected subcutaneously, or 1-2 drops are introduced into the conjunctiva of the eye). The disease commences within 5-7 days when a great number of *Borrelia* are found in the blood.

Treatment. Patients are treated with chlortetracycline, oxytetracycline, chloromycetin, albomycin, and streptomycin in combination with penicillin. Arsenic preparations (novarsenol, etc.) are not very effective.

Prophylaxis is ensured by measures aimed at extermination of ticks and rodents, early recognition of the disease, hospitalization of patients, and observance of individual prophylaxis (prevention of ticks attacking people).

There are about 20 independent nosologic forms of tick-borne relapsing fever (Spanish, Balkan, Iranian, Indian, African, North American, etc.) described, besides the Middle Asian form. They are produced by different serotypes.

Relapsing fever cases of various aetiology occur in many countries. In 1957-58, a total of 9,508 patients were registered in Africa, Europe, and Asia.

CAUSATIVE AGENT OF FUSOSPIROCHAETOSIS

Borrelia vincenti (H. Vincent, 1906) are Gram-negative spiral threads, 8-12 μ long and 0.3 μ thick, with 3-8 spirals. In association with fusobacteria (Fig. 123) they produce ulceromembranous angina and fusospirochaetoses (Plaut-Vincent angina, ulcerative stomatitis, noma, and other ulceronecrotic processes) in human beings.

Fig. 123. Borrelia and fusobacteria

Fusobacterium fusiforme (A. Veillon and A. Zuber, 1898) is a long spindle-shaped rod with pointed ends. It measures 5-16 μ in length and 0.5-1 μ in breadth. Threads up to 100-200 μ in length may occur which break up into short rods and cocci. The cytoplasm of fusobacteria is uniform or granular in structure. The cells are nonmotile and Gram-negative. The organisms grow at 37°C under anaerobic conditions on media which contain proteins (blood and serum). Smooth colourless or pale yellow-brown colonies are formed on solid media. Gelatin is not liquefied. Indole is produced. Glucose, saccharose, maltose, levulose, and, sometimes, lactose are produced, with acid formation. Fusobacteria are found in the mouths of human beings in ulcerative lesions, on the genital organs, and in cases of appendicitis.

These two species of conditionally pathogenic bacteria occur quite frequently in association in the form of various types of parasitocoenosis. Lowered body resistance facilitates activation and development of clinically manifested forms of fusospirochaetoses.

Treatment is accomplished with penicillin and novarsenol.

Prophylaxis comprises normal sanitary measures, e.g., care of the mouth, prevention of local and general cooling, systematic hardening of the body, timely treatment of inflammations in the nose and throat, tonsillitis, chronic rhinitis, and other diseases.

CAUSATIVE AGENT OF SYPHILIS

Treponema pallidum, the causative agent of syphilis, was discovered in 1905 by F. Schaudinn and E. Hoffmann.

Morphology. The organisms belong to the genus *Treponema*. They are spiral threads with 14-17 small and uniform convolutions (Fig. 124). The treponemas are 6-14 μ in length and 0.25-0.3 μ in breadth.

They are motile (capable of rotating, translational, bending, and undulating movement) and stain poorly with dyes. They are stained light-pink by the Romanowsky-Giemsa method as a result of the low nucleoproteid content in the cell.

Cultivation. *Treponema pallidum* is an extremely fastidious organism. It does not grow on ordinary media, but grows at 35°C under anaerobic conditions on media which contain ascitic fluid and brain tissue. Growth temperatures range between 34 and 40°C. The organisms are grown on medium consisting of 2 parts of 2 per cent agar, 1 part of ascitic fluid, and pieces of sterile rabbit kidney. The cultures are covered with paraffin or vaseline oil. *Treponema pallidum* grows readily on the chorioallantoic tissues of chick embryos and on rabbit serum to which pieces of brain tissue are added under a layer of vaseline oil.

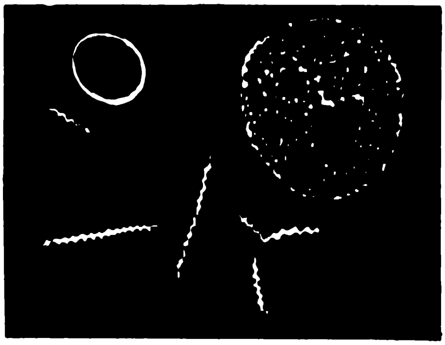


Fig. 124. Causative agent of syphilis in dark-field illumination

Pure-culture isolation of the treponema organism is extremely difficult. On prolonged cultivation, the organisms lose their virulence. Cultures adapted to nutrient medium are known as cultural as distinguished from tissue cultures which are pathogenic and are preserved in the laboratory by passage on rabbits. Cultural and tissue treponemas differ in their antigenic properties.

Antigenic structure and classification. The existence of serological types has not been ascertained. Some authors maintain that there are two varieties of the causative agent of syphilis: dermatotropic and neurotropic. However, it is doubtful whether such a differentiation is valid. *Treponema pallidum* displays no marked tropism. It affects various organs and tissues, including the nervous system. There is no doubt that different strains of *Treponema pallidum* exist, some of which are used in laboratory diagnosis as antigens in the Wassermann reaction. The cultural strains differ among themselves in a number of properties, e.g., changes in pH of the medium, degree of anaerobiosis, indole and hydrogen sulphide production, and reactions with carbohydrates. Many cultural strains cause haemolysis of human, sheep, horse, rabbit, and guinea pig erythrocytes. The organisms contain polysaccharides and lipid and protein complexes which possess very complicated antigenic properties and are highly specific.

Resistance. *Treponema pallidum* can survive for long periods at low temperatures in diseased tissues. It is destroyed in one hour

at 45-48°C, and in 15 minutes by a temperature of 55°C. It is sensitive to acids and other disinfectants and to desiccation.

Pathogenicity for animals. The organism is only slightly pathogenic for animals with the exception of monkeys. A number of authors obtained successful results by infecting rabbits through the cornea or testis. Laboratory infection of animals with syphilis has provided knowledge on problems of immunity, specific chemotherapy, and cultivation of the causative agent of the disease.

Pathogenesis and disease in man. Persons affected with the disease are sources of infection. The disease is transmitted via the genital organs and by contaminated dishes and other objects. The causative agent localizes at first in the mucous membranes of the genital organs and mouth and in the skin. At the site of penetration it multiplies and produces proliferative and destructive changes. Syphilis may also be transmitted through the placenta (congenital syphilis).

Three periods of syphilis are distinguished.

Primary syphilis develops after a variable incubation period of 2 weeks to 3 months (average 21-24 days). It is characterized by the formation of a primary syphiloma, a hard infiltrate with an erosion or ulceration on its surface, at the point where the treponema enters the body. The floor and edges of the ulcer are of a cartilaginous consistency (for this reason the lesion is known as *ulcus durum*, primary sclerosis, or hard chancre). Primary syphiloma is accompanied by the development of regional adenitis manifested by enlarged and hard lymph nodes. The first stage of the disease lasts for about 6 weeks.

Secondary syphilis is manifested by eruptions on the skin and mucous membranes and development of specific lesions in the internal organs, bones, and peripheral and central nervous systems. This period may vary from 2-3 to several years.

Tertiary syphilis is characterized by the production of papules, tubercles, gummas, or gummatous infiltrates in the skin, subcutaneous cellular tissue, internal organs, etc. The lesions have a tendency to produce necrosis. This period persists for several years.

In some cases, progressive paralysis or tabes dorsalis develops after 9-10 years. A large number of treponemas are present in the brain tissue during this period and cause deep organic changes in the central nervous system.

Immunity in syphilis differs considerably in character from that in other infections. The disease produces no insusceptibility. Individuals who have recovered from the disease may be re-infected. Immunity in syphilis is infectious, and characterized by cellular defence reactions (the lymphocytes produce lipolytic enzymes which cause lysis of treponemas). The presence of antibodies is no indication of body resistance.

A state of infectious allergy, a peculiar manifestation of body reactivity, is a characteristic feature of syphilis. During primary syphilis (hard chancre), the reactivity is lower. An increase in reactivity occurs most frequently during the later periods and is accompanied by deep changes in the tissues and organs.

The state of allergy may be revealed by the intracutaneous luetin reaction. Luetin is prepared from *Treponema pallidum* cultures or diseased tissues.

Laboratory diagnosis. Microscopic examination of ulcer, erosion, or papula discharge or material obtained by puncture of the regional lymph nodes is performed during the primary and secondary periods of the disease. The smears are stained by the Romanowsky-Giemsa or Burri's method or impregnated with silver by Morozov's method, and examinated with the dark-field microscope. *Treponema pallidum* must be differentiated from the nonpathogenic organisms of the same species which occur in the external genital organs (*Borrelia refringens*, *T. genitalis*) and mouth (*T. microdentium*, *T. mucosum*). These organisms are not as delicate in structure as the *T. pallidum* and differ in their mode of motility and number of spirals. Dark-field examination yields positive results in almost 100 per cent of the cases.

The *Treponema pallidum* immobilization test is of great value. It is based on the ability of the antibodies, present in patient's serum, to immobilize the treponema. A suspension of the treponemas, obtained from testis tissue of an infected rabbit, is mixed with a complement and the patient's serum under test. The mixture is placed into an incubation chamber for 30 minutes at 35°C in an atmosphere of 5 per cent carbon dioxide and 95 per cent nitrogen. The immobilization test is extremely specific and, as a rule, yields no falsely positive reactions.

The Wassermann and precipitin reactions are of great importance in the diagnosis of seropositive syphilis.

The Wassermann reaction should not be confused with the Bordet and Gengou reaction. In the latter, the complement is adsorbed by a specific antigen-antibody complex. In the Wassermann reaction, both the lipid extract of organs of the foetus infected with syphilis and lipids of healthy organs of various animals may act as antigens. Therefore, the presence of a specific antigen is not necessary for the Wassermann reaction.

The mechanism of the Wassermann reaction has not yet been ascertained. Profound changes take place in the tissues during syphilis and heterogenous complexes are produced. Disturbance of lipid metabolism in particular occurs. As a result, autoantigens are produced which cause the formation of the corresponding antibodies in the patient's blood.

According to another point of view, the amount of globulins increases during syphilis, but their degree of dispersion diminishes.

This enhances their reaction with lipid extracts of various animal organs. As a result, complexes of various size are produced, which may adsorb the complement. This theory explains to a certain extent the mechanism of nonspecific reactions. At present, however, treponemas are considered to be full-value antigens. Since there is no fundamental difference between the lipids of treponemas and those of animal organs, the antilipid antibodies produced by the treponemas on their penetration into the body are capable of reacting both with the treponema antigens and the lipids of animal organs in the precipitin and complement-fixation reactions.

Most probably the Wassermann reaction is caused by immunological and physico-chemical factors. This theory is corroborated by the fact that the complement-fixation reaction is more effective and sensitive when it is performed with syphilitic treponema antigens which have been obtained from the tissues of rabbits infected with *Treponema pallidum.*

Fifty per cent of cases with primary syphilis give a positive Wassermann reaction, but not earlier than 2-3 weeks after the appearance of the chancre or 5-6 weeks following infection. The reaction is positive in 90 per cent of cases with secondary syphilis and in 75 per cent of cases with tertiary syphilis, mainly among those patients who have undergone no treatment or received inadequate treatment. Newborn and breast-fed children who have contracted syphilis yield a negative Wassermann reaction. From 95 to 98 per cent of patients with progressive paralysis and from 50 to 70 per cent of those with tabes dorsalis give a positive Wassermann reaction. In children with congenital syphilis, the reaction becomes positive 2-3 months after birth.

The Wassermann reaction (together with clinical manifestations and precipitin reactions) is a method of control over the efficiency of treatment. It becomes negative $1^1/_2$-2 months after specific treatment of primary and secondary seropositive syphilis has been enacted. The reaction may remain positive over a long period of time (1-$1^1/_2$ years and longer) during tertiary syphilis. It should be borne in mind that a negative Wassermann reaction is not the sole criterion of recovery from syphilis.

The Wassermann reaction may be positive beginning from the eighth month of pregnancy, after delivery, during malaria, tuberculosis, some protozoal and viral diseases, leprosy, pemphigus, leptospirosis, and tumours. A positive reaction may also be produced during the menstrual period, following injection of heterologous sera, if alcohol, rich food, drugs, or anaesthetics were taken on the day before the blood had been collected, etc.

These nonspecific positive Wassermann reactions become negative when they are repeated once or twice with an interval of 10-15 days. The pseudopositive and positive Wassermann reactions may be differentiated by the sera titration method (a high titre is produced

during fresh secondary syphilis; syphilis patients who yield a pseudopositive reaction produce a low titre).

Precipitin reactions are widely used in syphilis diagnosis, i.e., the Sachs-Witebsky test (the citochol reaction) and the Kahn test. The serum is mixed with a special lipid antigen (an alcohol extract of various animal organs) and balsamic substances (cholesterol) are added. A positive reaction is demonstrated by the formation of small granules or flakes and a negative one by the appearance of a uniform turbidity.

Treatment. Penicillin and arsenic, mercury, bismuth, and iodine preparations are effective. A. Rosenblum in 1876 and J. Wagner-Jauregg in 1917 suggested carrying out pyrotherapy for patients with progressive paralysis by infecting them with malaria plasmodia obtained from monkeys. Infection with the causative agent of relapsing fever and sodoku was later carried out for this purpose. Pyrogenal in combination with penicillin and bioquinine is used as a pyrogenic agent at present.

Prophylaxis comprises timely recognition of syphilitic cases, adequate treatment, health education of the population, and improvement of living conditions. Liquidation of unemployment, inequality of rights for women, brothels, and primitive living conditions have led to a sharp decrease in syphilis incidence in the Soviet Union and the Socialist countries.

Endemic Arabian syphilis, known as bejel, is similar to syphilis. It differs from syphilis in that it involves the skin, mucosa, and bones but does not affect the cardiovascular and nervous systems. The disease is widespread in the rural districts of Arabian countries, prevailingly among children.

Frambesia (yaws) which is caused by *Treponema pertenue* is also a variety of syphilis. It occurs in tropical countries (Africa, Ceylon, South America, Central America, India, Indo-China, South China, Indonesia, and North Australia). The disease mainly attacks the local population which lives under difficult conditions. Frambesia is a typical social endemic disease. Syphilis and frambesia are capable of producing cross immunity.

Pinta, a disease produced by *Treponema carateum* which is a variety of *Treponema pallidum*, occurs in South America, Columbia, Central America, the West Indies, and, sometimes, in Cuba.

Treatment is accomplished with antisyphilitic preparations (penicillin with a prolonged effect, chlortetracycline, oxytetracycline, and chloromycetin).

PATHOGENIC LEPTOSPIRAE

The pathogenic leptospirae are responsible for zoonotic diseases which are subdivided into icteric and anicteric leptospiroses.

Icteric leptospirosis (leptospiral jaundice, Vasilyev-Weil disease,

leptospirosis icterohaemorrhagica) is caused by *Leptospira icterohaemorrhagiae* (R. Inada and Y. Ido, 1915).

The causative agents of anicteric leptospirosis (water fever, meadow-land fever, water-field fever, grippe-typhus fever, silt fever, harvest fever, seven-day fever, autumn fever, etc.) include several varieties and serological types of leptospirae: *Leptospira grippotyphosa* (S. Tarassoff, 1928), *Leptospira tarassowi* (S. Tarassoff, V. Kiktenko, E. Galperin, 1938), *Leptospira pomona* and many others described in a number of countries.

Morphology. The leptospirae are made up of small closely coiled spirals (from 12 to 18 per organism), and resemble a tightly wound spring with thick hooked ends. They consist of a cytoplasmic cylinder and a rigid axial thread and have secondary spirals which give them an S-like appearance (Fig. 125). Leptospirae are 6-9 μ in length (sometimes even 20-25 μ) and 0.25-0.3 μ in breadth. They are motile and capable of rotation, gliding, and swinging motions. Leptospirae of icteric and anicteric leptospiroses are identical morphologically. They can only be distinguished by their antigenic structure. The organisms stain poorly with aniline dyes and are stained light-pink by the Romanowsky-Giemsa method. After treatment of the smears with a mordant, the organisms stain more distinctly. They may also be demonstrated by Burri's method and by Morozov's silver impregnation method. Leptospirae reflect light poorly. Treatment with different stains is accompanied by a change in morphology of the organisms.

Fig. 125. Leptospirae of infectious jaundice

The leptospirae are pleomorphous. They may lose their ability to form C- and S-like forms. Pleomorphous forms may be seen in the same culture. The changes may be symmetrical when both ends of the organism are altered, or asymmetrical when only one of the end hooks is changed. Both long and short forms may occur. Morphological variations of the organisms appear quite early after isolation from the body.

Cultivation. Leptospirae grow in liquid and semisolid nutrient media which contain blood serum and on Vervoort-Wolff medium which consists of 1 g of peptone, 0.5 g of NaCl, 1,000 ml of tap water, 5-10 ml of Sørensen's phosphate buffer mixture, and 10 per cent of inactivated rabbit serum. The organisms also grow well in medium

consisting of haemolysed rabbit blood and 0.1 per cent meat-peptone agar with pH adjusted to 7.2-7.4.

The optimal temperature for growth is 28-30°C (growth temperatures ranging from 22 to 37°C). The organisms are grown under anaerobic conditions over 7-10 days, the cultures being covered with liquid paraffin or vaseline oil.

Antigenic structure and classification. On the basis of serological properties, leptospirae are subdivided into groups, types, and strains. D. Bergey distinguishes 7 groups and 20 serotypes, while G. Wildfur recognizes 44 serotypes. Eighty-eight leptospirae types and subtypes were known in 1962, 20 of which were isolated in the USSR. The antigenic properties of the organisms are determined by agglutination and lysis reactions. No soluble toxin is produced. Toxic substances are present only in living leptospirae which are parasitic within a human or animal body.

Resistance. The organisms withstand low temperatures and survive in water over a period of many months. They are very sensitive to desiccation and acids. Exposure to 56°C kills the organisms in 30 minutes. Leptospirae are rapidly lysed in bile and bile acids.

Pathogenicity for animals. *Leptospira icterohaemorrhagiae* is pathogenic for rats in natural conditions. Rodents of the *Muridae* family and cattle contract anicteric leptospirosis. More than 80 species of wild and domestic animals may acquire leptospirosis. Among laboratory animals, guinea pigs are susceptible to the causative agent of leptospiral jaundice. An intraperitoneal inoculation into these animals is followed by the development of cachexia, numerous haemorrhages, and jaundice in 2-3 days. The animals die 5-6 days after the onset of the disease. A great number of leptospirae are revealed in the organs of the guinea pigs, particularly in the kidneys and liver. The causative agents of anicteric leptospirosis are pathogenic for white mice and produce haemorrhagic skin lesions. Rubbing the organisms into the skin of guinea pigs or bathing in water reservoirs contaminated with leptospirae produces a clinical picture typical for the diseases and, sometimes, with manifestations of jaundice.

Pathogenesis and diseases in man. Rats are the source of leptospiral jaundice, discharging leptospirae into the surrounding environment (water, soil, objects, and foodstuffs) with the urine. Contaminated water and foodstuffs are transmission factors. Small rodents (the field mouse, meadow mouse, wild rats, etc.), cattle, swine, and dogs are sources and reservoirs of anicteric leptospirosis and discharge the organisms with the urine. Human beings acquire the disease after swimming in contaminated water, drinking contaminated water or milk from infected cows, and during hay-mowing and the tending of sick cattle.

In some countries which have a vast network of canals (Holland, Belgium, etc.), leptospiral jaundice is an occupational disease as

a result of people being in constant contact with water contaminated by rats. The same hazards occur during work in rice fields (Japan and Indonesia). Sick people are not infective and cannot be sources of infection for other human beings.

Leptospira icterohaemorrhagiae enters the host body by the gastrointestinal tract and by injured skin and mucous membranes. The disease is characterized by a sudden onset, high pyrexia, headaches, and pain in the muscles. The disease involves the central nervous system. Jaundice develops and the liver becomes enlarged and painful. Haemorrhagic eruptions, nose bleeding, and gastric and intestinal haemorrhages occur, and the spleen becomes enlarged. Nephritis and anuria are also observed. Relapses occur in many patients.

Bacteraemia which develops in the first days of the disease plays an important role in the pathogenesis of leptospiroses. At the end of the first week, the organisms accumulate in the liver, spleen, lymph nodes, and bone marrow. Then they penetrate the kidneys and are discharged with the urine for a period of 4-6 weeks. Toxic substances produced as a result of dissociation of leptospirae cause lesions in the cells of the liver and other organs. Parenchymatous and, partially, fat degeneration of the liver cells and oedema of the intercellular tissue occur. Focal haemorrhages are observed in the spleen, and manifestations of haemorrhagic nephritis are common.

The causative agents of anicteric leptospirosis gain entrance into the body through injured mucous membranes and skin, with foodstuffs, with water during swimming in contaminated water, and on contact with infective material. Small rodents (field mice) and cattle in whom a carrier state of long duration has been shown are reservoirs for the organisms.

The disease occurs during the spring-summer season.

Anicteric leptospirosis (water fever) is characterized by a sudden onset, fever, severe headache, and pains in the bones and muscles. The patient loses his appetite. Nausea and lassitude are observed. The spleen and liver are enlarged. A typhoid condition, mental depression, delirium, dulled consciousness, and insomnia occur. A pleomorphous rash with haemorrhages is seen on the body. Either there is normally no jaundice, or else its manifestation is relatively rare. The patient usually recovers from the disease, but sometimes it may be followed by severe complications, such as meningitis, vitreous opacity, iridocyclitis, loss of hair, abortion, etc.

Immunity. The disease produces a stable immunity the mechanism of which is associated with the presence of antibodies. Agglutinins and lysins in 1:50,000-1:150,000 titres are found in patients' serum 8 or 10 days after the onset of the disease. The antibodies persist for many years.

Laboratory diagnosis comprises the following methods:

(1) direct dark-field microscopy of thick films and smears prepared from citrated blood, spinal fluid, urine, and organs obtained at post-mortem examination;

(2) isolation of a haemoculture and urine culture. Blood is collected from the patient on the third or fourth day of the disease and 10, 20, and 40 drops respectively are inoculated into 3 test tubes, covered with vaseline oil, and cultivated at 28-30°C. The urine culture is isolated by inoculating urine on the second or third week of the disease;

(3) agglutination and lysis reactions (see Fig. 70) with convalescent sera and all leptospirae species performed from the moment the temperature falls and up to 2 months after recovery. Positive reactions with sera in dilutions not less than 1:400 are of diagnostic value;

(4) complement-fixation reaction;

(5) laboratory inoculation of guinea pigs with patient's blood (during the first days of the disease, before the appearance of jaundice). The animals are given an intraperitoneal injection with 2-3 ml of patient's blood. Two or three days later, the exudate and blood are examined for the presence of leptospirae.

Treatment. Penicillin, chlortetracycline, oxytetracycline, polyvalent antileptospirosis serum and leptospirosis gamma-globulin are used.

Prophylaxis. Leptospiral jaundice is prevented by observing general sanitary precautions and extermination of rats. Vaccination of the population is performed in some cases.

Control of anicteric leptospirosis is ensured by recognition of cases among cattle, treatment of water to render it harmless, prohibition of swimming in poluted water reservoirs, and extermination of rodents. Protective clothing of hands is recommended for individuals working with manure.

Prophylactic vaccination with vaccine prepared from a suspension of the more common types of leptospirae is carried out.

PATHOGENIC MYCOPLASMAS

The *Mycoplasma* bacteria remained unrecognized for a long period of time. E. Nocard and E. Roux isolated from the lungs the causative agents of peripneumonia and pleuropneumonia of cattle. These organisms are known as PPO (pleuropneumonia organism). Later, organisms responsible for pleuropneumonia-like diseases in humans were discovered and were called PPLO (pleuropneumonia-like organisms). Originally, they were grouped with the large viruses. D. Bergey (1957) included these organisms in the genus *Mycoplasma*, family *Mycoplasmataceae*, order *Mycoplasmatales*, class *Schizomycetes*.

Morphology. The main information pertaining to the size, shape, and structure of the organisms is given in the part of this book concerned with general problems (see pp. 49-50). *Mycoplasma hominis*, the species pathogenic for human beings, has a mycelial structure whose characteristic feature is the presence of short rod-like filaments measuring 2-5μ in length. Coccal forms have also been revealed under the electron microscope.

Cultivation. Mycoplasmas are aerobes and facultative aerobes. Their optimal growth temperature is 37°C. They produce a small zone of haemolysis on blood agar. On solid media the organisms produce granular growth and form round colonies measuring from 10 to 500μ after 3-7 days' growth. In broth cultures of mycoplasma turbidity and a finely granular precipitate are formed. The organisms are cultivated on media containing serum and ascitic fluid with the pH adjusted to 7.0-8.0 under aerobic conditions, and at 37°C.

Nutrient media containing 10 per cent carbon dioxide are used for cultivating some strains. Some mycoplasmas are obligate anaerobes. Their growth is stimulated by adding yeast extracts to the media. They grow on media which are devoid of serum but which contain 0.02 per cent of haemoglobin and 0.01 per cent of cysteine. Mycoplasmas multiply readily in the chorioallantois of chick embryos.

Fermentative properties. Many species of mycoplasma ferment glucose with acid formation. The causative agent of pleuropneumonia ferments glucose, maltose, dextrin, starch, glycogen, and, sometimes, levulose, galactose, and mannose.

Antigenic structure and classification. D. Bergey divides the genus *Mycoplasma* into 15 species. Mycoplasma pathogenic for human beings (*Mycoplasma hominis*) consists of two types: type 1 which is nonpathogenic for mice and type 2 which produces local abscesses in mice when injected subcutaneously. Mycoplasmas possess species- and type specificity.

Toxin production. Strains, isolated from mice, produce a thermolabile exotoxin. Mycoplasma filtrates are toxic for mice. A relatively thermostable toxin has also been isolated from the organisms.

Resistance. The majority of strains are killed by a temperature of 45-55°C in 15 minutes. The organisms are very sensitive to all disinfectants, supersonic vibrations, desiccation, and other physical factors.

Pathogenicity for animals. Mycoplasmas (*Mycoplasma mycoides*) are responsible for pleuropneumonia in cattle. Some varieties of this bacterium produce pleuropneumonia in goats. Mycoplasmas have been revealed during inflammatory processes in the genitalia (*Mycoplasma bovigenitalium*) and in agalactia of sheep and goats (*Mycoplasma agalactiae*). The organisms are also pathogenic for dogs, rats, mice, and birds.

Pathogenesis and diseases in man. *Mycoplasma hominis* is the species which has been most studied. It occurs in pleuropneumonia, inflammation of the genitalia, nonspecific urethritis, prostatitis, nongonococcal arthritis, trichomonal-like lesions, endocarditis, sepsis, and other diseases. It is possible that diseases caused by mycoplasma are widespread among human beings and animals, but the lack of adequate laboratory methods makes their detection a hazardous procedure. As a result of this, mycoplasmas have been classified with various groups of organisms in the past, including a group of viruses. It is assumed that mycoplasmas are conditionally pathogenic and produce diseases in man when his general body resistance is significantly low.

Mycoplasma pneumoniae is another representative of this genus of organisms. It is responsible for primary atypical pneumonia (PAP), haemorrhagic laryngitis, and vesicular inflammation of the tympanic membrane. In 1944, M. Eaton isolated a filterable agent from patients' sputum which caused pneumonia in cotton rats when inoculated through the nose. Later investigations carried out by R. Chanok, L. Haifflik, and M. Borrel verified the hypothesis that the Eaton agent belongs to the mycoplasmas.

Immunity. Following pleuropneumonia cattle, sheep, and goats acquire a stable immunity of long duration. Immunity in human beings is a problem still not studied and is probably associated with the general resistance of the human body.

Laboratory diagnosis. Morphological, cultural, biochemical, and biological examination methods are employed for differentiating mycoplasmas from other microorganisms. In particular, a rise in the titre of nonspecific cold agglutinins against human erythrocytes of group I and specific antibodies against the Eaton agent occurs in human serum during atypical pneumonia. Other differential characteristics of *Mycoplasma pneumoniae* include its growth on noncellular PPLO-Difco medium in the form of typical colonies, haemolysis of erythrocytes in the medium, fermentation of carbohydrates, and the occurrence of minute particles measuring 180-250 mμ in filtrates. *Mycoplasma pneumoniae* produces pneumonia in cotton rats. The organism exerts no cytopathogenic effect in chick embryo and tissue cultures.

Treatment. Chlortetracycline, oxytetracycline, streptomycin, and chloromycetin yield the best results. No therapeutic effects are obtained with sulphonamides, penicillin, and erythromycin.

Prophylaxis. There are no specific measures of preventing diseases caused by mycoplasmas. Prophylaxis amounts to preserving high body resistance.

L-FORM BACTERIA

The historical aspect of the discovery of L-forms and their main characteristics are discussed in this book in the part concerned with general problems (see p. 44). These bacteria are a peculiar form of bacterial variability and are characterized by the fact that the L-forms of various species possess common properties.

Morphology. Bacterial forms transform quite frequently into L-forms on exposure to ultraviolet and X-rays, cooling, phenol, bacteriophages, antibodies, amino acids, penicillin, and chloromycetin. This process is accompanied by changes in the chemical composition of the cells and blockade of synthesis of high-molecular compounds in the protoplast and cell wall, and particularly by the loss of diaminopimelic acid in the cell wall. The changes in the cell wall and protoplast are responsible for the transformation of the typical bacterial forms into spherical forms.

The L-forms of various bacterial species are similar in morphology. They occur as spherical structures, 10-20μ in diameter, and as branching, vacuolated, vesicular-shaped, submicroscopic granular (250-300 mμ), and filterable elements which measure 125-200 mμ. Branching in the form of large filaments occurs most frequently among Gram-positive bacteria (*Corynebacterium diphtheriae*) and less frequently among the Gram-negative organisms (*Salmonella typhosa*, etc.). The submicroscopic structures are capable of intense brownian movement. The presence of actively motile large spherical bodies and flagella has been described among some strains.

Pleomorphism is an essentially characteristic feature of L-form bacteria and is the result of disturbance of the correlation between growth and multiplication. Due to inhibition of the process of division, a surface swelling appears on the sides of many rod-shaped bacteria. This swelling grows intensively and a spherical body is formed instead of a typical bacterial cell. Other bacteria grow into large filaments, or form nonstructural granular masses and spherical-shaped protoplasts in the initial stage of morphogenesis.

L-form bacteria multiply by division and by producing submicroscopic forms. The latter transform into spherical bodies and more complex structures.

Cultivation. Irrespective of the species from which they originate, all L-forms possess common cultural characteristics. They grow slowly on semisolid (1.4 per cent agar) or semifluid (0.4 per cent agar) media. On semisolid media they produce small or very large typical L-colonies which either grow down into the medium or are suspended in it. The colonies are viscous and consist of two parts, the peripheral part which is on the surface and resembles a lace pattern and the central part which is denser and darker and

grows down into the medium. The small colonies normally do not reverse to bacterial colonies, or else do so very rarely, while reversion of the large colonies occurs more frequently.

L-form bacteria grow only on media which contain human or horse serum or ascitic fluid. They may also be cultivated on synthetic media consisting of salts, amino acids and vitamins in agar, the pH of the media being 7.6-8.0. The frequency of L-form production varies in different species from 1:26 in *Proteus vulgaris* to 1:20,000,000 in *Salmonella*.

Fermentative properties. The transition of normal bacteria into L-forms is accompanied by the disturbance of protein, carbohydrate, and mineral metabolism and decrease in enzyme activity. It is noted, however, that together with the loss or inhibition of enzyme activity, the L-forms acquire new biochemical properties not found in the initial strains (fermentation of urea, synthesis of flavine, cholesterol, etc.).

Antigenic structure and classification. Deviations in the antigenic structure occur as a result of significant changes in the chemical structure. The antigenic species specificity is preserved to a certain extent in the majority of cases.

Attempts have been made to classify the L-forms and to determine their taxonomic status. However, this problem remains unsolved. Most probably, the L-forms cannot be classified in any single genus or family for the reason that they are originally associated with those species from which they are produced as a result of variability or a definite cycle of development (R. Tulyan).

According to Tulyan's classification, the L-forms may be distinguished from the pleomorphic forms of the causative agent of pleuropneumonia and other mycoplasmas. He included all the described L-forms into the group of *Bacteriopneumonialis.* This designation emphasizes the bacterial origin of the L-forms and their resemblance to PPLO (mycoplasmas). Depending on the bacterial species from which each L-form is produced, the following organisms can be distinguished: *Bacteriopneumonialis proteus*, *Bacteriopneumonialis esonerichia*, *Bacteriopneumonialis salmonella*, etc.

Resistance. It has been ascertained that L-forms are extremely resistant to factors responsible for the transition of bacterial cultures into L-forms. For this reason nutrient media which contain penicillin are employed for cultivation in some cases.

Toxin production and pathogenicity for animals and man. The pathogenic properties of L-forms are an unsolved problem. The majority of data provide evidence that L-forms, irrespective of their origin, are nonpathogenic. Isolation of L-forms from the body is a comparatively rare phenomenon. L-forms have been found to be present in haemocultures in some cases of septic infections.

The production of highly pathogenic L-forms of pneumonia and typhoid fever bacteria has been ascertained in laboratory studies. The reversion of L-forms into bacterial forms is probably of great significance. This may take place when certain changes occur in the medium (e.g., when penicillinase is added). L-forms constantly occur among typical cells in cultures isolated from animals or human beings. The L-forms are similar to mycoplasmas but at the same time they differ significantly. Mycoplasmas possess an antigenic structure and are permanently pathogenic for some animal species.

RICKETTSIAE

In 1910, the American investigators H. Ricketts and R. Wilder discovered the presence of small oval-shaped nonmotile and bipolarly stainable microorganisms in the blood of patients with Mexican typhus (tabardillo) and in infected lice. In 1913, the Czech scientist S. Prowazek discovered oval-shaped and elongated bodies, readily stainable with the Romanowsky-Giemsa reagent, in the plasma and leucocytes of human beings suffering from typhus fever.

In 1916, the Portugese scientist E. da Rocha-Lima summarized the available literature and, on the basis of his own investigations over many years, came to the conclusion that typhus fever was caused by small pleomorphic organisms which occur in patient's blood and in the guts of infected lice.

Great attention was then, and is given at present to the problem of rickettsioses. Many scientists devoted their work to the study of this group of infectious diseases.

Rickettsiae belong to the class *Microtatobiotes*, order *Rickettsiales*, family *Rickettsiaceae*. Data on morphological, cultural, fermentative, antigenic, and toxic characteristics are presented in the corresponding chapters of Part One of this book.

The classification of rickettsiae is given on p. 53 and that of the main rickettsioses in Table 31.

The majority of rickettsiae are commensals. About 50 different types of rickettsiae have been revealed in the guts and salivary glands of plant lice, bedbugs, and ticks.

CAUSATIVE AGENT OF TYPHUS FEVER

Morphology. *Rickettsia prowazekii* is a pleomorphic organism. It may be spherical or, more frequently, it resembles dumb-bells in shape (Fig. 126). Rod-like and thread-like forms also occur (see Fig. 18). The organisms measure 0.5-1μ on the average, the maximum measurements being 0.3-0.8 and 0.7-2.0μ. The thread-like

The Main Types of Rickettsioses and Certain Diseases Caused by Organisms of the *Chlamydiaceae* and *Bartonellaceae* Families, Order *Rickettsiales*

Disease	Causative agent	Vector	Occurrence
Epidemic or louse-borne typhus	*Rickettsia prowazekii*	Body or head lice	In many countries of the world, particularly in those with a mild or cold climate
Endemic or murine typhus	*Rickettsia typhi*	Rat fleas and lice and, possibly, rat ticks	North and South America, the coasts of the Baltic, North, Mediterranean, Black and Caspian seas, Asia, Africa, North Australia
Tsutsugamushi fever	*Rickettsia tsutsugamushi*	*Trombicula akamushi*, *Trombicula schueffneri*	Japan, Taiwan, Indonesia, New Guinea, North Australia
Rocky Mountain spotted fever	*Rickettsia rickettsi*	*Ixodes* ticks *Dermacentor andersoni*, *Dermacentor variabilis*	The USA, Canada, Mexico, Brazil, Columbia
Marseilles or Mediterranean exanthematous fever	*Rickettsia conori*	Dog ticks *Rhipicephalus sanguineus*	The coasts of the Mediterranean, Caspian, and Black seas, India, Tropical Africa
North Asian rickettsiosis	*Rickettsia sibirica*	*Dermacentor nuttali*, *Dermacentor silvarum*, *Dermacentor pictus*	Siberia, The Far East, East, Transbaikal
North Australian rickettsiosis	*Rickettsia australis*	*Ixodes holocyclus*	Northern Queensland in Australia
Rickettsialpox or vesicular rickettsiosis	*Rickettsia akari*	*Allodermanyssus sanguineus*	Outskirts of New York, some localities in the USSR
Wolhynian or trench fever	*Rickettsia quintana*	Body lice	Occurred on various fronts during World Wars I and II (in Poland and on the Wolhyn) and occurs in Japan and China
Tick-borne paroxysmal rickettsiosis	*Rickettsia quintana*	*Ixodes ricinus*	Some regions of the Ukraine
Q fever	*Coxiella burneti*	*Ixodes* ticks	Australia, the USA, Europe, Asia Minor, the USSR
Trachoma	*Chlamydia trachomatis*	No vectors	India and other countries of Asia and Africa

Disease	Causative agent	Vector	Occurrence
Ornithosis	*Miyagawanella ornithosis*	No vectors	In all countries
Psittacosis	*Miyagawanella psittaci*	No vectors	South America, Australia and other countries
Atypical pneumonia	*Miyagawanella pneumonia*	No vectors	In all countries
Lymphogranuloma inguinale or venereum	*Miyagawanella lymphogranulomatosis*	No vectors	Subtropical countries (the disease does not exist in the USSR)
Bartonellosis	*Bartonella bacilliformis*	*Phlebotomus verrucarum, Phlebotomus noguchii*	Peru, Ecuador, Bolivia, Chile

forms may reach 40μ in length. Rickettsiae are Gram-negative and readily stain red with phenol fuchsin (see Fig. 127,*1*), by the Romanowsky-Giemsa method, and by impregnation with silver by Morozov's method.

Cultivation. Rickettsiae multiply mainly in the cells of the vascular and mucosa epithelium. For methods of cultivation see p.101.

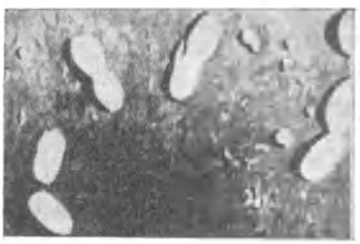


Fig. 126. Dumbbell-shaped rickettsiae

Toxin production. Rickettsiae contain a toxic substance which cannot be isolated from the organisms neither by filtration nor by centrifugation. The rickettsia toxin is a protein and is thermolabile, being destroyed at 66°C. An intraperitoneal or intravenous injection of a rickettsial suspension produces a lethal acute intoxication in guinea pigs in 2-24 hours.

Antigenic structure. *Rickettsia prowazekii* contains two antigens: a thermolabile antigen, specific for these organisms, and a thermostable antigen which is common to *Rickettsia prowazekii* and to the organisms of murine typhus. The soluble rickettsial antigens are identical to certain bacteria in their activity. In 1916, E. Weil and A. Felix isolated *Proteus OX19* from the urine of typhus patients. This organism is agglutinated by serum of typhus patients and convalescents. It has been ascertained that *Proteus* and rickettsiae have a polysaccharide hapten in common (the O-antigen). Other *Proteus* strains (OX2, OXK, and OXL) are also used in differential diagnosis of rickettsioses.

Resistance. Rickettsiae survive in dried and intact lice for as long as 30 days and in dry louse faeces for 6 days. They withstand 50°C for 15 minutes, 56°C for 10 minutes, 80°C for 1 minute, and 100°C for 30 seconds. All commonly used disinfectants (0.5 per cent phenol solution, 0.25 per cent formalin solution, etc.) are destructive to rickettsiae, killing them in 1-2 hours.

Pathogenicity for animals. Monkeys, guinea pigs, and white mice are susceptible to rickettsiae. Typhus fever with a clinical picture identical to that of the human disease may be reproduced in monkeys. An intraperitoneal infection of guinea pigs with blood of typhus patients produces fever and a 1-1.5° rise in temperature in 8-12 days. The organism occurs in the blood, internal organs, and in particularly large numbers in the brain. White mice infected through the nose under ether narcosis develop pneumonia.

Pathogenesis and diseases in man. In 1876, O. Mochutkovsky was the first to show by self-inoculation the infectiousness of the blood of typhus patients. He proposed that typhus is transmitted by blood-sucking insects. In 1909, Ch. Nicolle and collaborators verified this hypothesis in experiments on monkeys and demonstrated that typhus is transmitted by the body louse (*Pediculus vestimenti*).

The patient is the source of infection, and the body louse is the vector. After sucking blood of a typhus patient, the body louse becomes infective in 3-10 days or more frequently in 4-5 days. Rickettsiae develop in the guts of lice (in the epithelial cells of the mucous membranes) at a temperature of 30°C. The cells are destroyed as a result of accumulation of the organisms, and the rickettsiae appear on the skin, clothes, etc., being discharged with the faeces. Infection with typhus takes place not through the bite of a louse but by rubbing the organisms which are discharged during defaecation into the skin, or when the lice are crushed and the organisms penetrate abrasions and scratches on the skin and mucosa.

Infection with typhus may also occur via the respiratory tract. *Rickettsia prowazekii* enters the human body with the dust of dried faeces of the louse. This route of infection occurs among workers of disinfection service and laboratories where vaccines are prepared and where problems of typhus are studied in experiments on white mice.

Rickettsia prowazekii causes human typhus which is manifested by a febrile state and roseolous-petechial eruptions.

Typhus is a blood infection. During the pyrexia period, the causative agent occurs in the blood, blood cells, endothelium of vessels, skin, and brain, and in other organs.

Histopathological changes occur in the vascular system, particularly at the site of precapillary branching of the arteriolae. Swelling and intensification of proliferation of the sinus endothelial

cells result in thrombosis. Proliferative processes also develop in the tunica adventitia vasorum. Bead-like swellings of the vessel walls are produced (periarteritis nodosa). Numerous thromboses of the end branchings of the arterial system result in disturbance of tissue nutrition, and the death of cells, particularly those of the central nervous system. Several thousands of granulomas are found per 1 sq cm of brain surface.

In the postwar years, typhus has occurred sporadically and its clinical picture has changed. The disease acquires a milder course and there are almost no lethal cases registered.

Immunity. The disease leaves a stable immunity. The incidence of re-infections has increased in the past years and comprises about 50 per cent of the total number of cases of the disease.

Various explanations of the cause of re-infections have been put forward. The majority of authors maintain that typhus re-infections occur as a result of loss of immunity acquired following the first disease, whereupon the patient may become infected for a second time.

According to another point of view, typhus re-infections are recurrences of the first disease. (In the USA this form of typhus is known as Brill's disease.) They occur in rickettsiae carriers of long duration when they are exposed to unfavourable influences.

In 1934 H. Zinsser and in 1937-39 G. Parro advanced the hypothesis that immunity in typhus is nonsterile. In the Soviet Union K. Tokarevich, G. Mosing, P. Zdrodovsky et al. support the recurrence theory of typhus re-infections. These authors have put forward the results of numerous studies and laboratory research work in support of this conception.

Of definite interest is a report made by W. Price and collaborators stating that they had isolated rickettsiae from the inguinal lymph nodes of individuals who had suffered from typhus in the past. These individuals lived in a locality where no cases of typhus had been recently registered.

Laboratory diagnosis is based on serological methods of examination:

(1) the agglutination test with *Rickettsia prowazekii* (the Weil-Felix test with *Proteus OX19* has lately lost its practical importance due to its low specificity);

(2) the complement-fixation reaction.

Nobel's reaction (accelerated agglutination test), Minkevitch blood-drop method, the biological method (guinea pig inoculation), opsono-phagocytic reaction, indirect haemagglutination reaction, and the test for neutralization of rickettsial toxic substances are also employed.

It must be borne in mind that patients who have been treated with antibiotics may yield a low-titre reaction which shows no rise of the titre.

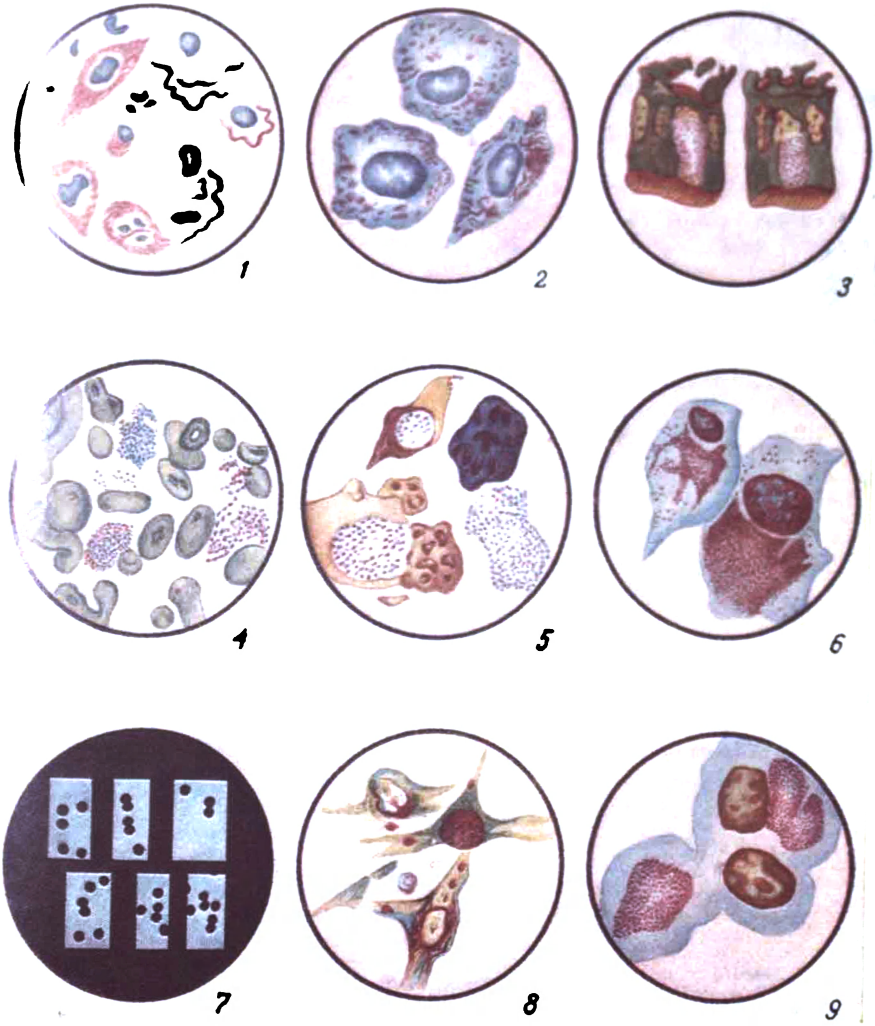


Fig. 127.

1—*Rickettsiae prowazekii*; *2*—*Rickettsiae mooseri*; *3*—causative agent of psittacosis; *4*—causative agent of ornithosis; *5*—causative agent of venereal lymphogranulomatosis; *6*—causative agent of trachoma; *7*—vaccinia virus; *8*—Negri bodies (red) within the Ammon's horn; *9*—inclusion bodies in blennorrhoea in the newborn

Treatment. The wide use of chlortetracycline, oxytetracycline, levomycetin, and synthomycin for treatment of typhus patients has led to a significant decrease in the death rate. No lethal cases have occurred in recent years.

Prophylaxis. The prevention of typhus comprises the following measures: (1) early recognition, isolation, and hospitalization of patients; (2) sanitation measures in the disease foci (fumigation); (3) registration and observation of all individuals who have been in contact with patients; thermometry of inhabitants of the disease foci for 25 days; systematic employment of delousing measures among the population and improvement of their education in hygiene; (4) specific vaccination as an additional precaution.

There are several types of vaccine. The Weigl vaccine is a phenolized emulsion of rickettsiae recovered from the guts of lice. Its preparation is difficult, particularly if large amounts are needed. A. Pshenichnov prepared a formalinized vaccine from rickettsiae obtained from larvae of lice which he fed with defibrinated blood through a membrane made from the skin of a corpse. The formalinized Cox vaccine is derived from rickettsiae grown on chick embryos. A live vaccine prepared from avirulent strains is very effective. It is a preparation of rickettsiae which have been cultivated on 7-day-old chick embryos. It is produced in dry form.

A formalinized deposited vaccine obtained from rickettsiae adapted to the lung tissue of white mice and a concentrated ether vaccine are most widely used.

Typhus was a formidable infection in the past. The morbidity rate increased sharply during national disasters (famine and wars). About 200,000 men of the Russian forces were attacked by the disease from which more than 40,000 of them died during the Russian-Turkish War (1758-75). It is known that there were vast epidemics of typhus during the Napoleonic wars. More than 2 million people contracted the disease from 1812 to 1814 in Germany alone. Vast typhus epidemics also occurred during World War I. In Serbia 50 per cent of the population (1.5 million) were attacked by typhus and 150,000 of the patients died in 1915.

The Civil War and economic dislocation gave rise to mass infection with typhus in Soviet Russia. More than 6 million people suffered from the disease during the period between January 1, 1918 and October 1, 1920.

The morbidity rate was also very high during World War II. The population of countries temporarily occupied by the German aggressors in particular suffered from the disease. Thus, more than 60 per cent of the total population of rural regions of Belorussia contracted typhus.

Epidemic typhus has been eliminated in the USSR as a result of a significant improvement of living conditions of the people and the practice of preventive measures.

The morbidity rate of typhus has decreased significantly in all countries. According to data furnished by WHO, 1,936 typhus patients were registered in 28 countries of the world in 1957, and in 1958 the corresponding number was 1,418.

CAUSATIVE AGENT OF MURINE TYPHUS

Rickettsia typhi (*R. mooseri*) was discovered in 1928 by H. Mooser. This organism (Fig. 127, *2*) is more pleomorphic than *R. prowazekii*. *R. mooseri* measure from 0.35 to 1.3μ. They are cultivated easily in chick embryo and remain viable in the external environment for long periods, particularly in desiccated form.

Monkeys and rabbits are relatively resistant. Infection of male guinea pigs by application of the infective material to the eye conjunctiva and mucous membranes of the nasal and mouth cavities produces a fever. The most characteristic symptom of intraperitoneal inoculation is the appearance of rickettsial periorchitis (the scrotal phenomenon). Rats and mice are very susceptible to *R. mooseri*.

Rats and mice are the main reservoirs of the causative agent in nature. They infect each other, the aetiological agent being transmitted by fleas, lice, and, probably, by ticks. Per oral infection is also possible.

Human beings contract endemic murine typhus from rodents. The causative agent enters the body through the mucous membranes of the eyes, nose, and mouth, injured integuments, and through the respiratory tract. It may also be conveyed with foodstuffs contaminated with the urine of sick animals and by contact (by rubbing in faecal matter of infected mice when scratching the skin). Human beings may also be infected by the bite of the rat tick Bdelonyssus bacoti.

Human murine typhus is usually endemic or sporadic in character. Depending on the epizootic state, local outbreaks of the disease may occur in some cases. The disease is seasonal, being more prevalent in August-November (the period of increased density and activity of rodents).

Human murine typhus is identical to epidemic typhus in many ways. The disease is characterized by fever and a rash on the face, chest, abdomen, back, palms, and soles. The rash is roseolous initially but later becomes papular. A petechial rash is rare.

The disease leaves a relatively stable immunity, and cross immunity to epidemic typhus is acquired.

Differentiation from epidemic typhus is made by carrying out parallel agglutination and complement-fixation reactions with *R. prowazekii* and *R. mooseri*. Male guinea pigs are infected for reproduction of the scrotal phenomenon.

Treatment of murine typhus patients is accomplished with chlortetracycline, synthomycin, and levomycetin.

Murine typhus control is ensured by extermination of rats and mice, prevention of rats penetrating into dockyards from incoming ships, protection of foodstuffs from rats, and extermination of rat fleas, lice, and ticks with DDT and chlorophos. Immunization of individuals living in endemic areas is practised in some cases.

CAUSATIVE AGENT OF MARSEILLES FEVER

Rickettsia conori was described for the first time in 1910 by A. Conor and A. Bruch. The organisms are rod-shaped and very pleomorphic. They are 0.3-0.4μ in breadth and 1-1.75μ in length. The rickettsiae grow well on outliving chick embryo tissues. Monkeys, guinea pigs, rabbits, rats, mice, and susliks are susceptible to the aetiologic agent of marseilles fever.

Marseilles fever is an endemic and seasonal disease (prevailing in summer). It is transmitted by the bite of the *Rhipicephalus sanguineus* tick. The organisms survive in the body of the tick at various phases of its development. It has been shown that ticks are capable of transovarial transmission. Ticks and dogs on which they are parasitic serve as the reservoirs of the causative agent. Furthermore, infection is possible through the conjunctiva of the eye by rubbing the rickettsiae into the mucous membranes.

The disease is accompanied by a pustular rash. At the onset of the disease the rash is roseolous or macular in character, but later it becomes maculopapular and, occasionally, secondary petechiae appear. In the majority of cases Marseilles fever assumes a benign course and there are no relapses.

Laboratory diagnosis includes the following methods: (1) complement-fixation reactions with patients' sera and *R. conori* (the most specific test); (2) isolation of the organisms from patients' blood, ulcerative skin lesions, roseola, and from ticks recovered from dogs. The blood or ticks ground in a mortar are injected intraperitoneally into male guinea pigs. The infected animals are examined for the presence of rickettsiae in the mesothelium of the affected vaginal coats of the testes. Intranuclear location of the organisms confirms the diagnosis of the disease.

Treatment is accomplished with chlortetracycline and oxytetracycline.

Prophylaxis is ensured by general measures of epidemic control. All domestic dogs and hounds are registered in endemic areas and treated with 10 per cent DDT ointment, and the places where they are kept are fumigated for extermination of ticks. Stray dogs are killed.

CAUSATIVE AGENT OF NORTH ASIATIC RICKETTSIOSIS

In 1938 Soviet scientists (M. Krontovskaya et al.) revealed rickettsial diseases, transmitted by ticks of the genera *Dermacentor* and *Haemophysalis*, in endemic sources in Western, Central, and Eastern Siberia, the Far East, Central Asia, and the Mongolian People's Republic.

The agent, *Rickettsia sibirica*, is morphologically identical with the organisms responsible for Marseilles fever. *R. sibirica* is rod-shaped, but thread-like rough forms may also occur. The organisms are grown on outliving chick embryo tissues and localize in the nuclei of the affected tissue and cells of the various organs.

The rickettsiae are pathogenic for monkeys, guinea pigs, rabbits, rats, and mice.

The disease is transmitted by ticks of the genus *Haemophysalis* which are parasitic on domestic animals and on large wild animals which live in the steppes and plains. They are most active in the spring, when they attack human beings and cause infection. The disease occurs in the season between April and October showing the highest incidence in May. The infection is prevalent among the rural population.

North Asiatic rickettsiosis is characterized by an enlargement of the lymph nodes and the appearance of roseolous or papular rash and haemorrhages on the chest, back, and inner surfaces of the extremities. The rash may also appear on the face, palms, and soles. It persists even after the temperature has fallen and leaves a pigmentation. Conjunctivitis and scleral injection are also noted. The disease assumes a benign course and gives no relapses.

Laboratory diagnosis is usually made by employing the complement-fixation reaction with patients' sera and antigens obtained from *R. sibirica*. The diagnostic titre of the complement-fixing antibodies is low (1:20-1:200). A positive reaction is usually obtained, beginning from the eleventh day of the disease.

Isolation of the causative agent by intraperitoneal infection of male guinea pigs with patients' blood collected in the initial stages of the disease is performed in special laboratories. The infected animals display fever and orchitis (the scrotal phenomenon), and the rickettsiae accumulate in the coats of the testis.

Prophylaxis is accomplished by individual protection of human beings from tick attacks and by complex measures aimed at extermination of ticks which are parasitic on domestic animals. These measures include the use of DDT, hexachlorocyclohexane, and chlorophos powders and disinfection of permanent and temporary living quarters.

CAUSATIVE AGENT OF TSUTSUGAMUSHI FEVER

This disease has been known for a long time, but its rickettsial aetiology was ascertained only in 1930 by M. Nagayo and collaborators. The causative agent *Rickettsia tsutsugamushi* (*R. orientalis*) is a small pleomorphic nonmotile bacteria-like organism which is identical to other rickettsiae of the tick group. The organism is parasitic within the cell, and is 0.3-0.5μ in breadth and 0.8-2μ in length. *R. orientalis* is Gram-negative. The organisms stain purple with the Romanowsky-Giemsa stain in impression smears and occur within the cytoplasm of mononuclear cells. A surface membrane and dense granules scattered in the cytoplasm can be seen distinctly under the electron microscope.

R. orientalis multiplies in mammal tissue cell cultures, chorioallantois, yolk sac, and agar tissue cultures. The organisms produce a toxin which kills white mice in several hours following injection.

The organisms are relatively labile to factors of the external environment but withstand a temperature of —70°C for long periods and are resistant to desiccation.

In nature *R. orientalis* occurs in the body of field mice and various rat species. The larvae of trombiculid mites (*Trombicula akamushi* and *Trombicula schueffneri*) are vectors of the organisms, the latter being transmitted by the transovarian route. Monkeys, rabbits, guinea pigs, mice, rats, cotton rats, and hamsters are susceptible to the organism. Infection results in the development of characteristic symptoms and pathoanatomical changes.

Human beings contract the infection when they are attacked by infected larvae of trombiculid mites. A fever of 2-3 weeks duration develops and a macular or maculo-papular rash appears. A small ulcer (an eschar) covered with a dark crust forms at the site of inoculation, and regional adenitis develops. The cardiovascular and central nervous systems become involved in severe cases and complications (pneumonia) are common. The death rate used to be very high (from 8 to 60 per cent) prior to the use of antibiotics. A sick individual is noninfectious. Tsutsugamushi fever is widespread in Japan, India, Indonesia, North Australia, and on islands in the Indian and Pacific Oceans.

Immunity is type-specific. The disease leaves insusceptibility to the particular species or strain for several years, but infection with other rickettsial strains results in second diseases.

In laboratory diagnosis the agglutination reaction with *Proteus OXK*, is employed. This reaction quite frequently yields a positive result at the end of the second week of the disease as a result of accumulation of agglutinins in the patients' blood. White mice are inoculated intraperitoneally with patient's blood collected during the fever period. The majority of infected mice die in two

weeks. Autopsy reveals characteristic changes and the presence of the causative agent in the exudate.

Treatment is accomplished with antibiotics of the tetracycline group. As a result, the death rate has decreased to zero.

Prophylaxis comprises measures aimed at extermination of mites and prevention of their attacking human beings. Chemoprophylaxis with antibiotics and vaccination have so far not proved to be effective.

CAUSATIVE AGENT OF RICKETTSIALPOX

Rickettsia akari was described by R. Huebner, W. Jellison, and C. Pomerantz in 1946. It is transmitted by the mite *Allodermanyssus sanguineus.*

Rickettsialpox is a general infectious disease with an incubation period of 10 to 12 days. Its characteristic feature is fever, and an abundant vesiculo-papular rash resembling that in chickenpox appears on the third or fourth day of the disease.

Rickettsia are revealed in patients' blood during the febrile period. They are highly pathogenic for white and grey mice, guinea pigs, and for white, grey, and cotton rats. The organisms are grown in chick embryos. They are intracellular and extracellular parasites and multiply readily in the lungs of white mice during intranasal infection.

The complement-fixation reaction with a purified and highly active antigen obtained from rickettsiae is employed for diagnosis. The disease occurs in the vicinity of New York, in some regions of the USA, in Africa and Ukraine (Donbas). It is registered all the year round but is somewhat prevalent during May and June. House mice and grey rats are reservoirs of the infection. The vectors (gamasid mites) are capable of transovarial transmission and as a result of this they are also reservoirs of the infection in nature.

Prophylactic measures against rickettsialpox are similar to those employed for the prevention of murine typhus (extermination of rodents and use of insecticides destructive to mites).

CAUSATIVE AGENTS OF PAROXYSMAL RICKETTSIOSES

1. **Wolhynian, or trench, fever.** The causative agent of this disease is *Rickettsia quintana* (Töpfer et al.), an extracellular organism parasitic in the gut of the louse. It is transmitted by the body louse from the reservoir in sick human beings. Wolhynian fever is an epidemic infection associated with pediculosis among the population. The disease takes a benign course with pain in the muscles and bones and relapses of fever. No rash appears. Laboratory diagnosis has not been worked out.

Wolhynian fever used to occur widely as outbreaks during World War I, in the Balkans, Syria, and Mesopotamia, and on various European fronts during World War II. The disease may be widespread in pediculous foci, but its presence is not always recognized by physicians.

2. **Tick-borne paroxysmal rickettsiosis.** The causative agent is *Rickettsia quintana*. The disease was described by N. Sirotinin et al. The vector is the *Ixodes ricinus* tick, and mites and rodents (field voles) act as reservoirs. The disease is a sporadic benign infection displayed by paroxysmal fever; no primary affect or rash is present. Cases have occurred in some localities of the Ukraine.

RICKETTSIA RESPONSIBLE FOR Q FEVER

Q fever is a disease which was first revealed among slaughterhouse workers in Australia in 1937 by E. Derrick. It was named by the first letter of the English word *query* (question, doubt). In 1939 F. Burnet and G. Freeman isolated the causative agent from patients' blood and urine.

Q fever is distributed in many countries throughout the world. Beginning from 1948, it has been registered in some regions of the Soviet Union.

Morphology. The aetiological agent (*Coxiella burneti*) is a small (0.25-0.5μ) lancet-shaped organism (Fig. 128) made up of a capsule, cytoplasm, and nuclear material. It may be coccoid, ovoid, rod-like, or thread-like. The presence of filterable forms has also been demonstrated. *Coxiella burneti* is nonmotile, Gram-negative, and stains readily with the Romanowsky-Giemsa, Morozov's, and Zdrodovsky's stains.

Cultivation. *Coxiella burneti* is grown by Cox's method in the yolk sac of the chick embryo. Large numbers of the organism accumulate in the sac and are used for preparing a diagnosticum and vaccines. Embryos of the first passage die in 9-12 days and those of subsequent passages, in 5-7 days.

Coxiella burneti displays no fermentative properties. The presence of a toxin has not been demonstrated. However, the organisms contain allergens which sensitize the body and cause the development of vasculitis and granulomas.

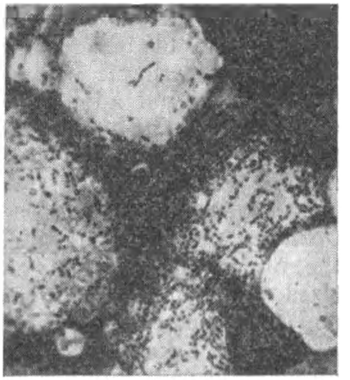


Fig. 128. The causative agent of Q fever

Resistance. The organisms survive in the external environment for long

periods, in sterile tap water for 160 days, and in whole sterile cow's milk at room temperature for 125 days. Pasteurization of milk does not destroy the organisms. *Coxiella burneti* can be well preserved in curds, yoghurt, and other milk products. In fresh meat of infected animals at 4°C they remain viable for 30 days, in frozen meat even longer, and in salted meat (10 per cent salt concentration) in a refrigerator for 5 months. *Coxiella burneti* withstands exposure to ultraviolet rays for periods from 1 to 5 hours and low temperatures for several months. The organisms remain viable in milk heated to a temperature of 90°C for more than one hour. They survive in dry tick faeces for $19^1/_2$ months and in dried-up urine and blood of sick animals for periods from several weeks to 6 months. Dried rickettsiae may survive in ampoules in a refrigerator vacuum for several years. The organisms live in a 1 per cent phenol solution for 24 hours and in a 0.5 per cent formalin solution for 4 days. They withstand exposure to gastric juice, ether, toluol, and chloroform.

Disinfection of the external environment and objects can be effectively carried out with a 5 per cent phenol solution, 3 per cent chloramine solution, 2 per cent calcium chloride solution, 2 per cent formalin solution, 10 per cent sodium hydroxide solution, and 0.2 per cent solution of activated chloramine.

Pathogenicity for animals. Cows, goats, sheep, dogs, horses, donkeys, mules, sand voles, birds, and ticks are sources of the causative organism of Q fever in foci. The animals discharge the causative agent with the milk, urine, placental tissues, amniotic fluid, and faeces.

Guinea pigs are experimentally injected intraperitoneally with 3-5 ml of blood obtained from patients at the height of the febrile period. Fever lasting from 1 to 6 days develops in the infected animals 5-13 days after inoculation. The patients normally recover from the infection, and death from this disease is relatively rare. Post-mortem examination reveals involvement of the spleen and lungs (haemorrhagic pneumonia), and, sometimes, rickettsial peritonitis. Regional adenitis occurs when the causative agent is inoculated into the thigh of a guinea pig. The infection may be reproduced experimentally also in mice, white rats, hamsters, and rabbits.

Pathogenesis and disease in man. On entering the animal body, *Coxiellae burneti* penetrate the cells of the tissues and organs of the reticuloendothelial system, blood, and lymph. The phagocytized organisms do not undergo lysis (incomplete phagocytosis), and they multiply in the leucocytes, producing septicaemia.

Infection takes place through the bite of *Ixodes* ticks. The latter discharge a vast number of the agents with the faeces, and the organism enters the human body with food, water, and by an air-droplet route.

Coxiellae burneti are extremely invasive, and penetrate easily through the mucous membranes, respiratory tract, and injured skin. Infection has been shown to be associated with work in slaughter-houses, the meat industry, and dairies. Humans contract the disease during the processing of wool, when they are in the vicinity of farm yards and dairies, and when they ingest raw milk and dairy products obtained from sick animals. Disease incidence prevails in April and May. Direct transmission from patients is rare, but their sputum must be disinfected since it may be the source of infection for healthy persons.

The clinical picture of the disease depends, to a certain extent, on the character of the primary localization of the causative agent. An intracutaneous infection results in local skin lesions in the form of hard erythematous spots which remain for a period of 10 days. Fever and septicaemia occur only in some cases. Infection through the upper respiratory tract (through the nose) is followed in 3 days by fever and typical changes in the lungs where dense pneumonic foci appear. Q fever is characterized by pleomorphic clinical symptoms. Three forms of the disease can be distinguished: pneumonic, febrile or influenzal, and meningoencephalitic. Each form produces characteristic distinctive symptoms. The onset of the disease is sudden and follows an incubation period of 7-28 days. It begins with a sharp rise of temperature up to 39-40°C accompanied by chills, severe headache, myalgia, lassitude, and insomnia. Death from this disease is a very rare occurrence.

Immunity. The disease in human beings is followed by a stable and lasting immunity. In animals the infection becomes chronic (lasting for several months), and this provides evidence that the disease produces low-grade immunity.

Laboratory diagnosis comprises the following methods:

1. Agglutination test with a specific antigen prepared from *Coxiella burneti* cultures. The test yields a positive reaction beginning from the second week of the disease, maximal titre values appearing during the third-fifth week. The appearance of a positive reaction in patients' sera diluted in a ratio 1:10-1:16 is diagnostic. An agglutination test with animal sera is of definite value for retrospective detection of possible sources of Q fever. For differentiation of a typical case of the disease from convalescence and latent infection, the test must be repeated during the course of the disease;
2. Complement-fixation reaction with a specific *Coxiella burneti* antigen (more sensitive and specific);
3. Allergic test which is strictly specific;
4. Subcutaneous or intratesticular inoculation of the material under test into guinea pigs;
5. Infection of guinea pigs by an intraperitoneal injection of 3-5 ml of blood taken from the patient during the febrile period, followed by inoculation of an emulsion prepared from the spleen

of a passaged guinea pig into a chick embryo. The same method is used for recovering rickettsiae from ticks and animals.

Treatment. Chlortetracycline, oxytetracycline, levomycetin, and synthomycin are used.

Prophylaxis. Preventive measures include systematic disinfection of premises where cattle and sheep are kept, especially during calving and lambing time. Milk obtained from infected dairies must be boiled because pasteurization does not destroy *Coxiella burneti*. Patients are transferred to hospital, and their excretions are disinfected.

Vaccination with vaccine prepared from *Coxiella burneti* cultivated on chick embryos and killed in formalin is carried out in places with a high disease incidence.

BARTONELLA

In 1905 A. Barton discovered the presence of organisms in the erythrocytes of sick human beings, which were later grouped in the family *Bartonellaceae*, order *Rickettsiales*.

Bartonellae are small pleomorphic organisms which occur as straight or curved rods 0.25-0.5μ in breadth and 1.0-3.0μ in length. Within the erythrocytes the organisms are arranged in groups, sometimes occurring in chains and forming the letter V. The Romanowsky-Giemsa reagent stains them a deep light-blue colour. Flagella can be demonstrated in the electron microscope.

Bartonellae grow on blood agar and allontoic fluid of the developing chick embryo. Their optimal growth temperature is 26-28°C.

The organisms are pathogenic for field voles, rats, and some other rodents. They also cause disease in guinea pigs, dogs, cattle, lampreys, pikes, etc.

Bartonella bacilliformis is pathogenic for human beings. Infection results in bartonellosis which is characterized by involvement of erythrocytes and cells of the spleen, lymph nodes, liver, bone marrow, vascular endothelium, and almost all internal organs.

Infection is conveyed by the bites of *Phlebotomus verrucarum* and *Phlebotomus noguchii* sandflies which transmit the causative agent from sick persons or latent carriers. It is possible that rodents and domestic animals serve as reservoirs of bartonellae. Bartonellosis is mainly spread among people living in extremely poor sanitary conditions in close contact with domestic animals and rodents.

Bartonellosis is accompanied with severe haemolytic anaemia. The incubation period lasts from 1 to 40 days. The temperature rises to 39-40°C and remains at this level for 10-30 days. The main symptoms of the disease are headache and severe pain in the muscles and joints. Erythrocytes decrease in number to 1-2 million per ml and the haemoglobin value to 20-30 per cent. The first stage

of the disease which is accompanied by haemolytic anaemia is followed in 1-2 months by the second stage, during which warts resembling those in yaws appear. The death rate varies from 10 to 40 per cent during the first stage (at the time of severe anaemia), and there is no case fatality during the stage of wart formation. Immunity of a high degree is produced following the disease.

Diagnosis is made on the basis of the clinical picture (fever and the development of haemolytic anaemia in the first stage and the appearance of warts in the second stage). During the acute period blood films stained by the Romanowsky-Giemsa method are examined, blood is inoculated on Noguchii serum agar, and an agglutination reaction with patients' serum is carried out (a 1:20 titre is diagnostic).

Treatment is conducted with antibiotics (chloromycetin, penicillin, tetracycline, and streptomycin) which mainly render harmless bacteria responsible for secondary infection (*Salmonella typhimurium*) and to a lesser degree, the bartonellae. Blood transfusions which accelerate the recovery process and raise body resistance to secondary infection are very effective.

Prophylaxis amounts to extermination of sandflies, protection from their bites, and improvement of sanitary conditions at work and in everyday life.

CAUSATIVE AGENTS OF ORNITHOSIS, VENEREAL LYMPHOGRANULOMATOSIS, AND TRACHOMA

Organisms of the family *Chlamydiaceae*, order *Rickettsiales*, are spherical, ovoid, or rod-shaped and can be seen under the light microscope. Most of them cannot pass through bacterial filters, and some of the organisms form intracellular clumps. They are composed of nucleoproteids, lipids, and carbohydrates. The organisms display antigenic properties similar to those of bacteria. They possess complex enzyme systems which are sensitive to those antibiotics which are inhibitory towards a large number of bacteria. Some authors consider this group of organisms to be large viruses.

CAUSATIVE AGENT OF ORNITHOSIS

Ornithosis (Gr. *ornis*—bird) is a disease of many birds. The aetiological agent was discovered in 1933 by K. Meyer.

Morphology. The causative agent *Miyagawanella ornithosis* is circular in form, 250-300 mμ in size, and occurs intracellularly (in the cytoplasm) or extracellularly. In smears and microscopic sections obtained from organs, the organisms can be seen as clumps which are surrounded by a capsule. The Romanowsky-Giemsa or Morozov's stain reveals the presence of the organisms in the cells of the reticuloendothelial system or outside the cells when the latter disrupt (see Fig. 127, *3*, *4*).

Cultivation. The causative agent develops in chick embryos, the body of white mice, tissue cultures, and in tumour cells. Four stages of its development can be distinguished: (1) no visible forms present; (2) appearance of large structures; (3) increase in the number of small particles; (4) development of typical forms. Within chick embryo cells the development cycle is completed in 72 hours.

The toxigenicity of the organism can be demonstrated by the fact that exposure to formalin or ether does not deprive it of anti-

genic properties. It is probable that the toxic substances are contained in the thermolabile (protein) fraction which is destroyed at a temperature above 60°C.

Antigenic structure. The organism possesses two antigens, a thermostable and a thermolabile antigen. The former withstands heating to a temperature of 135°C and is composed of polysaccharides which are common to the ornithosis-psittacosis-lymphogranulomatosis group. The thermolabile antigen is destroyed at 60°C and contains a protein.

Resistance is very high. The causative agent survives for long periods in a desiccated state and at low temperatures. In a frozen state it remains viable for 2 years at a temperature of —70°C. Infected tissues remain contagious for several weeks when kept at 4°C. Heating to 60-70°C kills the organism in 10-15 minutes. The causative agent is sensitive to exposure to the common disinfectant solutions (chloramine, phenol, and formalin).

Pathogenicity for animals. Parrots, pigeons, chickens, and many other species of birds are very susceptible to the causative agent of ornithosis. The disease is also easily contracted by white mice, rats, rice-meadow mice, guinea pigs, rabbits, and monkeys. The resulting infection is accompanied by sepsis and involvement of the internal organs.

Pathogenesis and disease in man. After entering the human body, the aetiological agent invades the blood and produces bacteraemia which lasts for a week or even longer. As a result of the various cycles which it undergoes within the tissues and organs, disturbances of metabolism appear and intoxication and allergy develop. The presence of a pneumonic focus unaccompanied by dyspnoea, cough, and pain in the chest is characteristic of ornithosis. X-ray examination at the height of the disease reveals marginal lobular pneumonia. Resorption of the exudate is slow.

The disease is spread by birds (sick or carriers), e.g., domestic and wild pigeons, ducks, chickens, turkeys, and wild birds. Adult birds recover from the disease, while large numbers of the young die. The causative agent is discharged with the excrements which become sources of infection for birds and human beings.

Man usually acquires the infection through the air when he inhales contaminated dust or down. He may also contract the disease cutting poultry, cleaning cages, and taking care of birds. The infective agent reaches the mucous membranes from dirty hands. Infection from person to person by air-droplet route is also possible.

The clinical picture of ornithosis is similar to that of atypical pneumonia, influenza, typhoid fever, typhus fever, lobar and catarrhal pneumonia, tularaemia, and Q fever.

Immunity following the disease is relative and of short duration. Repeated infections occur, especially among laboratory workers.

The mechanism of body defence is associated with the presence of antibodies.

Laboratory diagnosis. The causative agent is detected in the sputum and blood during the first days of the disease. It is present in the blood up to the fifth-seventh day of the disease and in the sputum to the twenty first day. A maximal registered period of pathogen discharge in the sputum is 8 years. The organism is recovered from the lungs, spleen, and exudate during post-mortem examination. Ornithosis can be demonstrated by microscopy with Morozov's method and by carrying out the complement-fixation reaction with paired sera (at the beginning and at the end of the disease). The causative agent is isolated from patients' blood and serum by intracerebral inoculation of white mice.

The organisms may be recognized in smears made from the spleen and liver of laboratory animals infected intraperitoneally and in the microscopic sections of their brain. It may also be isolated by inoculating the yolk sac of a chick embryo. The allergic test with ornithine gives a good result.

Treatment. Chlortetracycline, oxytetracycline, tetracycline, and streptomycin are prescribed.

Prophylaxis of ornithosis is ensured by a series of measures: early diagnosis, isolation and hospitalization of patients (they are sent to isolation departments and placed in isolated wards or compartments). The attending personnel must wear masks and regularly disinfect their hands with a 0.5 per cent chloramine solution. Patients are discharged from the hospital only on complete recovery. In the disease focus the premises and patients' clothes and objects of individual use must be disinfected. Patients' sputum is disinfected with a 4 per cent chloramine solution, 5 per cent calcium chlorate solution, 0.5 per cent solutions of potassium hydroxide or sodium hydroxide, and 5 per cent solution of lysol. Sick birds are killed and their nesting places are disinfected. In view of a high ornithosis incidence among pigeons, it is necessary to double veterinary control measures and restrict or ban pigeon breeding in towns or near poultry farms.

Psittacosis (Gr. *psittakos*—parrot) is a variety of ornithosis. The causative agent *Miyagawanella psittaci* produces an infectious disease in parrots and human beings. Psittacosis in parrots is characterized by rhinitis, enteritis, and wasting diarrhoea and is usually lethal. The disease occurs in South America, Australia, and other countries.

Human psittacosis is accompanied by bronchitis and bronchopneumonia. Infection is spread by an air-droplet route. A carrier state of more than 10-year duration is left by the disease in some cases.

Laboratory diagnosis, treatment, and prophylaxis are the same as in ornithosis.

CAUSATIVE AGENT OF VENEREAL LYMPHOGRANULOMATOSIS

The organism *Miyagawanella lymphogranulomatosis* (K. Gamma, 1924) measures 200-250 mμ and is coccal shaped. It occurs intra- and extracellularly in pairs, groups, and chains or forms compact clumps, 1-10μ in size (see Fig. 127, *5*). In chemical structure and staining properties it is similar to the causative agent of ornithosis. It is sensitive to penicillin, sulphonamides, and some antimony preparations. The organism does not survive in 50 per cent glycerin.

The organism is responsible for venereal lymphogranulomatosis (Nicolas-Favre disease) in humans. The infection is transmitted by the genital route. It occurs in hot subtropical countries. This disease does not occur in the USSR.

Laboratory diagnosis is made by microscopy, the smears being stained by Morozov's method or with the Romanowsky-Giemsa stain. The complement-fixation reaction and allergic test (Frei test) are of diagnostic value. Intracerebral inoculation of white mice resulting in lethal meningitis is also used.

Treatment is accomplished with chlortetracycline, oxytetracycline, penicillin, and sulphonamides.

CAUSATIVE AGENT OF TRACHOMA

Chlamydia trachomatis is a coccal-shaped organism (see Fig. 127, *6*), 200-500 mμ, and sometimes 800 mμ in size. With the Romanowsky-Giemsa method it stains light-blue or violet. L. Halberstaedter and S. Prowazec (1907) discovered inclusions measuring about 10 μ in the cytoplasm of conjunctival epithelial cells. The organism passes through bacterial berkefeld filters. It displays marked tropism and multiplies only in the cells of the squamous epithelium of the conjunctiva and cornea.

Monkeys are susceptible to the causative agent of trachoma. Intraconjunctival inoculation produces experimental trachoma, similar to the disease in humans. The reservoir of the causative agent is man.

Disease in humans is accompanied by blepharo-keratoconjunctivitis. There is chronic inflammation of the conjunctiva and cornea with hyperplasia of adenoid tissue and hypertrophy of the follicles which resemble transparent granules.

In severe cases the hypertrophied follicles give the conjunctiva the appearance of frog spawn. Later the follicles cicatrize. Trachoma is spread by patients using common towels, washing in a communal basin, and by dirty hands and flies.

Laboratory diagnosis of trachoma is made by detecting inclusions in conjunctival epithelial cells.

Treatment is performed successfully with antibiotics (tetracycline, erythromycin, and synthomycin) and sulphonamides which together with other measures make up a complex course of treatment. A 1 per cent chlortetracycline unguentum gives a very good therapeutic effect.

Prophylaxis comprises timely recognition and proper treatment of patients, a dispensary service in disease foci, observance of hygiene in working and living conditions, and improvement of the welfare and cultural level of the population. Disease rate of trachoma is very high in India (where from 80 to 90 per cent of the population is suffering from the disease) and in other countries of Asia and Africa.

VIRUSES

Information on the size, morphology, structure, nature, and cultivation of viruses as well as on the pathogenesis and immunity of viral diseases has already been dealt with in the general part of medical microbiology.

VIRUSES CONTAINING DESOXYRIBONUCLEIC ACID

SMALLPOX VIRUS

Smallpox was known in Egypt 3,000 years B. C. Ruffert found traces of the disease on the skin of an Egyptian mummy. In China and India people have suffered from smallpox from time immemorial. From the works of ancient Chinese physicians there is evidence that the infection was spread to Arabia. In the VI century it spread to Europe (France and Italy). The infectious character of smallpox was ascertained in the IX century by the Arabian physician Rhazes (850-923) and by Avicenna, the eminent physician and philosopher of Central Asia.

The disease swept throughout large areas, becoming endemic, during the Crusades (between the XI and XIII centuries). In 1507 it was introduced into America by the Spanish. Smallpox became widespread in the XVII and XVIII centuries. More than 60 million people died from the infection in the XVIII century.

In Russia smallpox was reported in 1427 and swept widely throughout the country in the XVI century. In 1610 the disease spread to Siberia where an extremely severe epidemic occurred causing the death of more than 50 per cent of the population. In Russia in the prerevolutionary period from 100,000 to 150,000 people contracted the disease and about 20,000 of them died.

Morphology. G. Guarnieri in 1892 discovered the presence of intracellular bodies in histological sections of the cornea of a rabbit inoculated 2-3 days previously with the smallpox virus. These

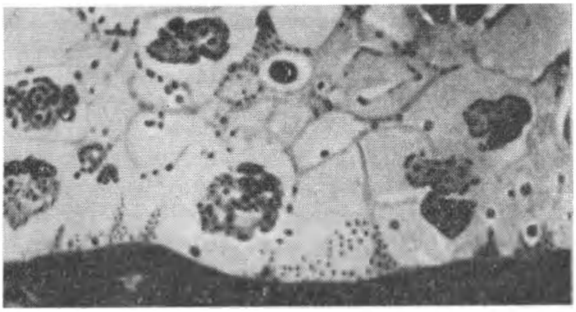


Fig. 129. Guarnieri bodies

inclusion bodies measured from 1-4 to 10 μ in size and were spherical or falciform (Fig. 129).

In 1906 E. Paschen found viral particles measuring from 125 to 180 mμ in the smallpox vesicles (Fig. 130). In the electron microscope they are seen in the form of cubes (bricks) with smoothed-out edges and are 227-305 mμ in size.

In 1926 M. Morozov elaborated a method for staining the smallpox virus, which is now employed in all countries.

Cultivation. The smallpox virus may be isolated during any stage of the disease and even during convalescence. Blood (serum and leucocytes), skin lesions, and the contents of vesicles and pustules are examined. The material obtained is diluted in common salt solution and treated with penicillin and streptomycin. After this, 0.2 ml of the preparation is introduced into the chorioallantoic membrane of at least 5 developing chick embryos and into test tubes containing tissue cultures. The remaining material is inoculated into blood agar for detecting the presence of pathogenic microflora. The infected chick embryos are incubated at 35-36°C for 3-4 days. They are inspected every day and the dead embryos are removed. HeLa and Hep-2 strains are used as tissue cultures on which multiplication of the virus and its cytopathogenic effect may be demonstrated.

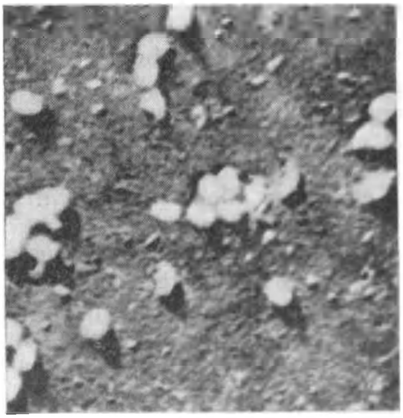


Fig. 130. Smallpox virus (in an electrone microscope)

Antigenic structure and clas-

sification. The smallpox virus displays no variants. In morphological, cultural and immunogenic properties the vaccinia (see Fig. 127, *7*) and smallpox viruses are identical and produce cross immunity. The current method of inducing immunity to smallpox in human beings by vaccinating them with the cowpox virus is based on this principle.

Resistance. The smallpox virus is resistant to phenol solutions and desiccation. It survives for months in dried crusts of smallpox lesions and easily withstands high concentrations of glycerin (50 per cent) and low temperatures. The virus is susceptible to the action of light and high temperatures. It is instantly destroyed at 100°C and perishes in 1 hour at 60°C. Potassium permanganate kills it in 70 minutes. The virus quickly loses its virulence at room temperature.

Pathogenicity for animals. The majority of animals (cows, calves, rabbits, guinea pigs) and birds are readily susceptible to the pox virus. The virus initially localizes in the skin and the upper respiratory tract mucosa.

Pathogenesis and disease in man. The source of infection is the patient. Infection takes place by the air-droplet route and through contact with infective material. The causative agent is spread by dust particles and certain objects (clothes, bedclothes, and dishes) and also when the patient talks, coughs, and sneezes.

The pathogenesis of smallpox has not been studied sufficiently. It is known that a virus which possesses extremely manifest dermatotropic properties is present in the blood at the time of the disease. It also affects the mucous membranes and other tissues and organs. Virusaemia is frequently accompanied by bacteraemia caused by streptococci, staphylococci, and pneumococci.

Smallpox is characterized by fever, the appearance of a rash and development of pustules and scars on the skin.

After the prodromal period and a fall in temperature, the true rash makes its appearance on the face, trunk, and extremities. Initially the rash is papular but later it becomes vesicular and pustular.

The smallpox vesicles are multilocular and their translucent content gives them an appearance of a pearl which has a mother-of-pearl hue and is surrounded by a narrow red areola. The smallpox pustules have a crater-like depression at the top.

During the suppuration stage there is a rise in temperature, the general condition of the patient deteriorates considerably, and he partially loses consciousness and suffers from extreme pain due to increased pressure in the pustules. This period is quite frequently accompanied with secondary infection (staphylococcal and streptococcal). In the majority of patients deep pustules leave scars (pockmarks).

According to its severity, smallpox is divided into the following forms: a mild form without rash and varioloids (smallpox occur-

ring in vaccinated individuals), a form of average severity (with isolated or confluent rash elements), and a grave haemorrhagic form. Depending on the severity of the disease, the mortality rate ranges widely from 0 to 100 per cent. It is 15 to 20 per cent on the average, but increases to 100 per cent for the haemorrhagic form. Among the mild forms and varioloids the mortality rate is low and normally there are no fatal cases.

Immunity. Smallpox leaves a lasting immunity and re-infections are extremely rare. Agglutinins, precipitins, lysins, and complement-fixing and virus-neutralizing bodies are found to be present it the blood of individuals who have suffered from the disease and in those who have been vaccinated.

Laboratory diagnosis includes:

1. Demonstration of the Paschen granules in the vesicle and pustule contents by employng virusoscopy of smears prepared according to Morozov's method. The virusoscopy (A. Borrel) and silver impregnation methods are employed in morphological examination and, in certain cases, in diagnosis of smallpox and other viral diseases.

Luminescent virusoscopy is a promising and specific method. Only specimens free of lipids can be examined by this method as some of the fluorochromes produce luminescence in the presence of fatty components of normal tissues.

Thin virus smears are dried at room temperature for 24 hours, washed in distilled water for 10 minutes, and stained with a 0.1-0.2 per cent solution of a fluorescent dye for 15 seconds.

Luminescent microscopy is also performed for demonstrating viruses in contaminated tissue sections, employing specific antiviral sera treated with fluorochromes.

2. Inoculation of the rabbit's cornea with material obtained from the vesicle of the smallpox patient (reproduction of Paul's phenomenon), (Fig. 131). In 24-48 hours ulcerous keratitis develops. The enucleated eye is submerged into an alcohol solution of mercuric chloride. Raised whitish granules which have a central depression are seen on the surface of the cornea. Guarnieri bodies are found in corneal sections. The virus is also isolated in the chorioallantoic membrane of a chick embryo and in tissue cultures. Complement-fixation and haemagglutination-inhibition reactions are carried out.

Fig. 131. Paul's phenomenon

In smallpox and chickenpox differentiation the following points are taken into account: smallpox vesicles are mul-

tilocular while chickenpox vesicles are unicamerate; the smallpox rash appears gradually, first on the face, then on the trunk and finally on the extremities, while the chickenpox rash appears almost simultaneously on the face, scalp, trunk, and extremities. In smallpox changes in the rash elements take place successively (papules, vesicles, pustules, and drying up of the latter) whereas in chickenpox the rash elements are pleomorphic and there is no uniformity in the time of their appearance. The complement-fixation test, rise in antibody titre, and haemagglutination-inhibition reactions are characterized by marked specificity.

The differential diagnosis of varioloid and a number of other diseases presents great difficulties, particularly in communities where a large number of individuals have been subjected to smallpox vaccination.

In varioloid there is no succession in the appearance of eruption, and fever lasts for about 3 days (with no second wave). The general symptoms are not typical. The rash may appear from the third day of the disease, the elements varying in number; the initial small spots quickly change into papules and vesicles; the red areola surrounding the vesicles may be very narrow; quite frequently the pleomorphic character of the rash is similar to that in chickenpox; the pustules dry up on the seventh or eighth day; the vesicles are not confluent.

Some symptoms of varioloid are identical to those of drug disease which produces a smallpox-like exanthema. Varioloid is characterized by a prodromal period, high temperature prior to the appearance of the rash, and hardness of the vesicle bed. The rash elements are arranged concentrically and on parts of the body covered with clothes. The rash also appears on the palms and the soles of the feet.

In view of the fact that clinical diagnosis of varioloid is a matter of difficulty, virologic examination of the contents of papules, vesicles, pustules, crusts and scabs, and of mucosa discharge is obligatory. It is also necessary to carry out all other tests used in laboratory diagnosis of typical forms of smallpox.

Treatment. At the present time, a specific gamma-globulin together with symptomatic and pathogenetic drugs is used for treating patients. It is obtained from sheep vaccinated with the vaccinia virus. In India good results have been obtained with a derivative of methylisatin-tiosemicarbazone-marboran, which significantly reduced lethality among contacts. Secondary infection is treated with antibiotics (penicillin, chloromycetin, streptomycin); oxychlortetracycline renders a good effect.

Prophylaxis. The general measures include timely diagnosis, hospitalization of smallpox patients, and disinfection of the focus.

Vaccination. Inoculation against smallpox was known in the East many years before Christ. From time immemorial inoculations were performed in India, Iran, and Georgia by means of variolation (inoculation with infective

material derived from smallpox patients). This method was introduced into European countries in the eighteenth century. However, variolation was frequently accompanied by infection with other diseases, with the result that smallpox inoculation differed but little from naturally acquired smallpox.

E. Jenner's discovery was a turning point in the problem of effective smallpox control. He found that peasants in England, who milked cows affected with cowpox, developed pox vesicles on their hands; the vesicles suppurated within several days, dried up and cicatrized. Individuals who had suffered from cowpox did not contract smallpox or acquired it in a mild form. E. Jenner studied this problem for 25 years. He accumulated much data, performed a great number of investigations, and on May 14, 1796, conducted an experiment in public. He inoculated the hand of an 8-year-old boy, James Phipps, with the contents of a pustule on the hands of a dairymaid, Sarah Nellms, infected with cowpox. One-and-a-half month later he inoculated the boy with the contents of a pustule of a smallpox patient. The boy did not contract the disease. He was infected again in several months time, and 5 years later he was inoculated with infective material from a smallpox patient. The boy proved to be insusceptible to the disease.

In 1801 doctor E. Mukhin, for the first time in Russia, vaccinated Anton Petrov, a boy from an orphanage, according to Jenner's method. In honour of this vaccination carried out for the first time in the country the boy was named Anton Vaccinov.

After Jenner's discovery the contents of vesicles in vaccinated children were used as inoculation material for a long period of time and vaccination was performed with "humanized lymph". However, such vaccination was dangerous since it was sometimes accompanied by infection with syphilis, erysipelas, and other diseases. The necessity of elaborating a new method for obtaining the vaccine led A. Negri to improve Jenner's method in 1842. He proposed to use a vaccine derived from calves. In 1866 O. Muller used glycerin for conservation of the vaccinia virus.

The smallpox vaccine contains the natural virus of cowpox, which possesses antigenic properties common to the smallpox virus, and is capable of producing a lasting immunity with no manifest general disease. There are indications of the vaccine strain being a hybrid of the smallpox and cowpox viruses.

The smallpox vaccine is prepared by artificial cutaneous infection of calves. For increasing the immunogenicity, the vaccinia virus is previously passaged through rabbits and as a result of this lapinus is obtained (Fr. *lapin*—rabbit). The latter is used for infecting calves. Before inoculation the skin of the calves is shaved clean and treated so as to exclude all foreign microflora. After this, cross-shaped incisions are made with a special scarificator and the lapinus virus is rubbed in. In 5 or 6 days vesicles form on the skin, and are then scraped off. The virus-containing material is collected with a special spoon into a glass jar which contains glycerin. It is left at room temperature for several days and then placed in a refrigerator at 2-5°C for 2 or 3 weeks so as to inactivate all foreign microflora. The scrapings are reduced to powder, and after determining the vaccination dose and testing for innocuousness the preparation is produced for use. At present, only a dry smallpox vaccine is produced which remains active for a year. It is applied by the cutaneous method.

In recent years an ovovaccine has been prepared. It is obtained from chick embryos inoculated with the virus. It is more profitable and is not inferior in quality to the vaccine prepared from calf's detritus.

On April 10, 1919, V. I. Lenin signed a decree issued by the RSFSR Council of People's Commissars enforcing compulsory vaccination against smallpox in Soviet Russia.

In the USSR children are vaccinated from the age of 3 months. Revaccination is performed at 4, 8, 12, and 18 years of age and every other five years thereafter.

Individuals who have been in contact with a smallpox patient are injected with anti-smallpox immune gamma-globulin. The specific gamma-globulin is prescribed for treatment and prophylaxis of postvaccinal complications.

In the USSR smallpox became sporadic beginning from 1928, and in 1936 this disease was exterminated. At the same time, in some countries and, particularly, in colonial countries it is widely distributed to this day. According to data furnished by the World Health Organization, 2,217,647 smallpox patients were registered over a period of 11 years (between 1951 and 1961) and 375,928 between 1960 and 1964. Over 100,000 cases of smallpox were registered in 1963 in forty countries throughout the world (India, Indonesia, Pakistan, the Congo, Brazil, and individual cases in Europe).

ADENOVIRUSES

In 1953 W. Rowe and collaborators discovered a group of viruses occurring in a latent state in the majority of human beings. These viruses are characterized by their cytopathogenic effect in relation to adenoid and tonsillar tissue cells, and for this reason they are known as adenoviruses (adenopharyngoconjunctival viruses).

Morphology. Examination by electron microscopy shows the viruses to be 70-90 mμ in size. The virion consists of a protein outer membrane and 252 capsomers 7 mμ in size. Adenoviruses have a polygonal hexahedral structure.

Cultivation. Adenoviruses grow in tissue cells of human beings, monkeys and other animals. Most susceptible are subinoculated epithelial cells HeLa, KB, Hep-2, etc., in which the cytopathogenic effect is relatively manifest. The nutrient media must be devoid of human or animal sera since the latter quite frequently contain antibodies against adenoviruses. The inoculated tissue cultures are incubated at 37°C for 14 days. Adenoviruses are nonpathogenic for laboratory animals and do not grow on chick embryo membranes. The process of cell degeneration consists of two phases. During the first phase changes in the cells are induced by toxin-like factors (initial phase of degeneration), while in the second phase viruses multiply within the nucleus and cytoplasm (final phase of degeneration). Inclusion bodies form within the nucleus. They consist of virions which produce aggregates of a crystalline structure. Adenovirus multiplication is accompanied by accumulation of excess lactic acid in the tissue culture.

Antigenic structure. Adenoviruses possess two antigens: a group antigen and a type-specific antigen. The former is demonstrated by the complement-fixation reaction and the latter, by the neutraliza-

tion test. Thirty types are known, 18 having been isolated from human beings and 5 from monkeys.

Resistance. Adenoviruses easily withstand thrice-repeated freezing and thawing. They resist desiccation by lyophylization and are insusceptible to ether. At a temperature of 56°C they are killed in 30 minutes.

Pathogenicity for animals. Animals are insusceptible to infection with adenoviruses under usual conditions, and, as a rule, inoculation has been unsuccessful. Piglets, born and grown in conditions of an aseptic external environment, display bronchopneumonia in 4 days following inoculation with serotypes 1, 2, 5, and 6 adenoviruses adapted to pig kidney tissue cultures. Adenoviruses of serotypes 12 and 18 cause malignant tumour growth in infected newborn hamsters.

Pathogenesis and diseases in man. Adenoviruses are responsible for acute respiratory diseases, virus pneumonia, contagious rhinitis, conjunctival fever, epidemic keratoconjunctivitis, acute follicular and membranous conjunctivites, and gastroenterocolitis. Respiratory diseases are most frequently caused by serotypes 3, 4, and 7. Type 8 is the main causative agent of epidemic keratoconjunctivitis. Of especial interest are membranous conjunctivites which are produced by serotypes 2 and 3 and are characterized by persistent fever, manifest catarrhal condition of the respiratory tract, and formation of membranes on the eye conjunctiva. Such conditions are quite frequently mistaken for diphtheria of the eyes. It has been noted in recent years that the clinical picture of the disease does not strictly depend upon the type of adenovirus. One and the same type may produce a wide variety of forms of the disease. The ability of various serotypes to produce one and the same disease has also been ascertained.

Immunity is associated with the presence of antibodies which are produced as a result of an acute and latent infection. The majority of newborn infants possess passive immunity which they lose at the age of 6 months. Susceptibility prevails at the age from 6 months to 5 years. Children older than 5 years of age possess antibodies and rarely contract adenoviral diseases. A relatively low disease incidence among adults is due to immunity acquired following an acute or asymptomatic form of the disease. The immunity is type-specific in character.

Laboratory diagnosis. It is extremely difficult to recognize adenoviral diseases by their clinical symptoms. They have much in common with a number of diseases of viral and bacterial origin. Adenoviruses isolated from patients and carriers can be differentiated from viruses of influenza, herpes, and poliomyelitis by laboratory methods. Adenoviruses cause cell degeneration in HeLa tissue cultures and delayed degeneration of fibroblasts in human embryonal tissue (Fig. 132) with formation of intranuclear inclusion bodies. The virus obtained is identified by serological reactions. The group

antigen is demonstrated by the complement-fixation reaction, and the serotype is determined by the neutralization test. To exclude the presence of a latent form of the infection paired sera are examined for a rise in the antibody titre. A fourfold increase in the antibody titre during the convalescent period as compared to that during the onset of the disease is diagnostic of adenoviral infection.

Treatment. The diseases are irresponsive to treatment with sulphonamides and antibiotics. These drugs are used for prevention and treatment of secondary infection caused by pathogenic and conditionally pathogenic bacteria. Patients with toxicosis are given injections of glucose and plasma. Hormonotherapy, oxygen therapy, blood transfusions, and ascorbic acid are also recommended.

Prophylaxis. The general antiepidemic measures have little effect. Current observations have shown that vaccination renders the desired effect. A formalinized vaccine is employed in the USA. It contains mineral oil which serves as an adjuvant. The vaccine is introduced subcutaneously or intramuscularly. R. Huebner has prepared a live vaccine which is given per os in gelatin capsules. Adenovirus vaccines are prepared from several types of viruses responsible for the most frequently occurring diseases (types 3, 4, and 7). However, specific prophylaxis of adenoviral infections is still under investigation. It is possible that it will be accomplished with compound preparations containing antigens against both bacterial and viral infections of children.

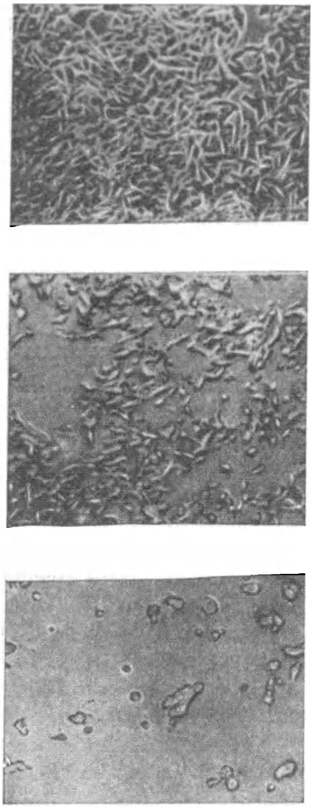


Fig. 132. Cytopathogenic effect of viruses

top—noninfected cells; *middle*—initial phase of degeneration; *bottom*—end phase of degeneration

HUMAN HERPES VIRUS

The virus was discovered by A. Löwenstein (1919) and W. Gruter (1920). Its size ranges from 130 to 233 mμ. The virus is coccal in shape and may be demonstrated by the virusoscopical method of Morozov. It forms granular intranuclear inclusion bodies in the epithelial cells.

The herpes virus is cultivated on the chorioallantoic membrane of the chick embryo on which it forms inflammatory necrotic foci.

It grows well on embryonal lung and kidney tissues of human origin and in cultures of HeLa cells, Detroit-6 cells, etc.

The virus contains two antigens: the V-antigen, which is found in the amnion and in the allantoic fluid and is tightly bound to the virus particles, and the S-antigen (soluble), which is revealed in the chorioallantois and in the tissue cultures on which the virus is grown.

The herpes virus is of low resistance. It dies at a temperature of 52°C. Desiccation at 90°C kills the virus in 30 minutes. It is susceptible to the action of 0.5 per cent formalin and 1 per cent phenol solutions, potassium permanganate, ether, chloroform, alcohol, and other disinfectants. It persists for a long time in a 50 per cent glycerin solution.

In natural conditions the virus does not produce infection in animals. Inoculation into the cornea of a rabbit, guinea pig, monkey, cat, mouse, and rat results in the development of keratitis. Introduction of the virus into the brain tissue gives rise to encephalitis. Skin lesions may also be reproduced in laboratory animals. Acidophilic inclusion bodies form in the nuclei of epithelial and nerve cells.

In human beings herpes is accompanied by epithelial degeneration and focal changes in the prickle-cell layer. The cells become larger and separate. Multicellular giant cells are produced as a result of amitotic cell division. A serous exudate discharged into the epidermis separates the transformed cells, thus forming a vesicle filled with exudate and epithelial cells. The presence of acidophilic intranuclear inclusion bodies is observable in the degenerate cells.

Primary and recurrent forms of herpes can be distinguished. Primary herpes is the result of infection by direct contact or by the air-droplet route. The disease usually occurs in the form of herpetic fever, and, less frequently, as herpes simplex. Herpetic fever brings about an increase of temperature to 39-40°C, severe headache, meningeal symptoms, vomiting, hyperaemia of the conjunctivas, and inflammation of the lymph nodes. On the following day a vesicular eruption usually appears on the lips, the temperature falls, and the disease takes the course of herpes simplex.

Herpes simplex, as a rule, is an acute condition with a sudden onset of pruritus and a burning sensation followed by hyperaemia, oedema, and herpetic vesicles developing on the border of the skin and mucous membranes, i.e., on the lips, in the nose, under the tongue, on the internal surfaces of the lips and cheeks, and on the genitalia. The skin lesions dry up and leave no marks.

Herpes recurrence is frequently encountered with certain diseases (malaria, influenza, acute catarrhal conditions, lobar pneumonia, meningitis, intoxications, psychic disorders), traumas, and cooling. In some cases herpes recurs during the menstrual period, with faulty diet and in the presence of other disturbances in the normal condition of the body.

No postinfection immunity is produced, and this accounts for the frequent recurrences and long-term virus carriage.

Diagnosis can be made from clinical symptoms. The presence of the virus may not serve as a diagnostic criterion as virus carriage is widespread among adults (from 70 to 90 per cent).

The virus is isolated by infecting the chorioallantoic membrane of the chick embryo, in which inflammatory necrotic foci are produced. Keratitis is easily reproduced in rabbits, guinea pigs, mice, and rats by inoculating the vesicle contents into the cornea, and encephalitis can be reproduced by an intracerebral infection.

Treatment is symptomatic. Severe cases call for prescription of vitamin B, and antibiotics are given to prevent secondary infection. Powder, zinc ointment, and an alcoholic solution of brilliant green are used. Autohaemotherapy is recommended in cases with recurrent forms of herpes, and X-ray therapy when there are recurrences in the same area.

Measures of prophylaxis have not been elaborated. During outbreaks of the infection in children's institutions and maternity hospitals the patients are isolated and quarantine is enforced.

CHICKENPOX (VARICELLA) VIRUS

The causative agent was discovered in 1911 by H. de B. Aragao and in 1917 by E. Paschen.

Morphology. The virus has an irregular spherical shape and is from 150 to 200 mμ in size. It forms intranuclear inclusion bodies in the epithelial cells and can be demonstrated by Morozov's stain.

Cultivation. The chickenpox virus grows well in the cells of human skin grafted onto the chorioallantoic membrane of a chick embryo. Examination of human skin epithelial cells reveals the presence of intranuclear inclusion bodies. The virus may also be grown successfully on amnion and kidney tissue cultures of human origin.

Antigenic structure and classification. The chickenpox virus is closely related to the virus of herpes zoster and yields a positive complement-fixation reaction with serum of patients suffering from this disease. It possesses no properties in common with the smallpox virus and differs from the latter with respect to immunology.

Resistance. The virus remains viable for a month in glycerin. It is unstable in the external environment and dies quickly, and for this reason disinfection is not carried out in cases of this disease.

Pathogenicity for animals. Only human beings are naturally susceptible to the disease. Animals do not contract chickenpox, and laboratory animals are not susceptible to the virus.

Pathogenesis and disease in man. The patient is the source of infection. The causative agent is spread by the air-droplet route. The patient is infectious from the last days of incubation and to the time the crusts fall off. Since the virus is extremely sensitive to

the action of environmental factors, infection takes place only in closed premises (apartments, child and medical institutions).

The skin, parenchimatous organs, and the brain are all involved in chickenpox. The epithelial cells become dystrophic and die. Intranuclear eosinophilic inclusion bodies are observable in the affected cells. Necrosis of the epithelial cells and accumulation of interstitial fluid lead to the development of vesicles. The epithelial eruption in chickenpox is pleomorphic. Erosions and small ulcers form on the mucous membranes.

The incubation period lasts from 2 to 3 weeks, and is followed by rise of temperature, chills, general lassitude, loss of appetite, and, sometimes, by vomiting and diarrhoea. Quite frequently, a scarlatina- or measles-like rash appears one or two days prior to the true eruption. It disappears in a few hours or in 1-2 days. The presence of vesicles followed by ulcers in the mouth and fauces is a characteristic feature of chickenpox. True eruption is accompanied by a rise in temperature and pruritus and appears in several times; the development of lesions is not uniform. Eruption ceases on the fifth day of the disease. Chickenpox takes a more severe course in adults. Uncomplicated cases of chickenpox normally terminate in recovery. Some cases become fatal due to secondary infection.

Immunity. The disease is quite rapidly followed by immunity which remains for life. Re-infections are extremely rare. Complement-fixing antibodies and agglutinins against the chickenpox virus may be demonstrated in blood of convalescents.

Laboratory diagnosis. Chickenpox is usually recognized by its clinical picture and on the basis of epidemiologic data. Laboratory diagnosis includes virusoscopy of vesicle smears and examination of histological preparations for the presence of intranuclear inclusion bodies. The isolated virus is identified by complement-fixation and virus neutralization reactions in tissue cultures. The size and shape of the virus and its pathogenicity for rabbits are taken into consideration for differentiation from smallpox. The chickenpox virus causes no lesions in the rabbit cornea.

Treatment and prophylaxis. There is no specific therapy. Antibiotics are prescribed for prevention of secondary infection. The lesion areas are treated with a 1 per cent alcoholic solution of brilliant green or with a 10 per cent potassium permanganate solution. The patient is not hospitalized but isolated at home until the crusts fall off. In medical institutions all child contacts are injected with gamma-globulin or serum of adults. Child contacts who have not suffered from chickenpox in the past are not allowed to go to children's institutions for 3 weeks.

HERPES ZOSTER VIRUS

The virus was isolated in 1944 by E. Goodpasture and K. Anderson. In the electron microscope the virus is seen to be similar in shape

to the chickenpox virus. It is rather difficult to differentiate these viruses by their morphological properties and size.

The virus is grown in tissue cultures on the chorioallantoic membrane of a chick embryo. Intranuclear inclusion bodies are found in the affected skin cells. Cytopathogenic changes and the presence of intranuclear inclusions identical to those in chickenpox are revealed in human embryonal tissue cultures.

The viruses of herpes zoster and chickenpox are identical in antigenic structure and produce cross agglutination and cross immunity. Chickenpox has been known to occur in children from families with cases of herpes zoster among adults. However, cross immunity is not produced in all cases. Sometimes individuals who had previously suffered from chickenpox contracted herpes zoster. Some authors regard herpes zoster as chickenpox in individuals of older age.

Lesions in the prickle-cell layer, inflammatory infiltration in the derma, formation of intranuclear and intracytoplasmic inclusion bodies in the affected cells, inflammatory infiltration in the nervous tissue with lymphocytes and plasma cells, and haemorrhages in the nerve ganglions followed by degeneration of the ganglionic cells in the nerve fibres are of essential importance in the pathogenesis of herpes zoster. The degenerative changes which take place in the peripheral nerves are of a secondary character and depend upon the involvement of the ganglions and dorsal roots.

The disease is characterized by the appearance of vesicles over a limited area of skin surface. The vesicles are usually distributed along the course of intercostal nerves and eruption is accompanied by a burning sensation, pruritus, neuralgia, and sometimes by a rise in temperature. The vesicles contain a clear fluid. Eruption is pleomorphic. In a number of cases the lesions may become confluent and resemble a continuous girdle (thus the term zoster which means a girdle or encircling structure).

The clinical picture is diverse both in the character of eruption and in generalization of the process. There can be an extremely severe complication when the eye becomes involved (iritis, ulcerous keratitis, retinal detachment, and haemorrhagic effusion in the camera oculi anterior). The spinal ganglions, posterior horns, and the paravertebral sympathetic ganglions may also be affected; lymphocytosis, increase in albumin content, and increase in pressure occur in the cerebrospinal fluid. Such conditions as pleuritis, pneumonia, cerebrospinal meningitis, tuberculosis, intoxications, injuries, excessive chilling, and other factors facilitate infection with herpes zoster.

No age group is entirely free from herpes zoster, but the disease is less frequent among children younger than 10 years of age.

The disease leaves a high-grade and lasting immunity, re-infections and recurrences being rare. Complement-fixing antibodies and agglutinins may be demonstrated in the blood of convalescents.

Herpes zoster is recognized by its clinical manifestations. Laboratory diagnosis comprises virusoscopy of the vesicle contents.

Treatment is symptomatic. The affected areas are treated with an alcoholic solution of brilliant green. Amidopyrine, phenacetin, antipyrine, pantopon, morphine, novocain nerve block, or novocain iontophoresis are prescribed for relieving pain. Antibiotics of the tetracycline group and penicillin are recommended for the prevention of secondary infection.

Prophylaxis is ensured by isolation of chickenpox and herpes zoster patients and by observing quarantine requirements.

VIRUSES CONTAINING RIBONUCLEIC ACID

INFLUENZA VIRUS

The viral aetiology of type A influenza was ascertained in 1933 by W. Smith, C. Andrewes, and P. Laidlaw. These authors proved that filtered nasopharyngeal washings obtained from influenza patients in the early stages of the disease were infective. T. Francis and T. Magill discovered the virus of type B influenza in 1940.

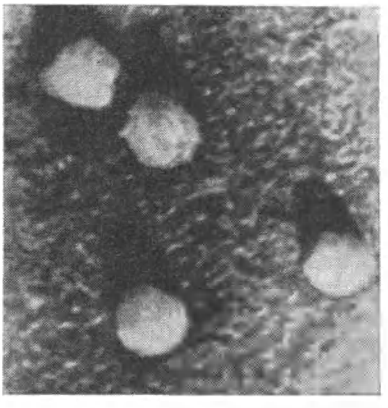


Fig. 133. Influenza virus

Morphology. The viruses are bean-shaped or rounded organisms (Fig. 133) measuring from 70 to 123 mμ in size.

The influenza virus contains an enzyme which is destructive to the receptor apparatus of the respiratory tract mucosa.

Cultivation. The influenza virus grows on the chorioallantoic membrane of the chick embryo, in monkey kidney and human embryo tissue cultures, etc. In chick embryo cultures the influenza virus occurs in the form of threads.

Antigenic structure and classification. Three types of the influenza virus can be recognized, A, B, and C. Type A comprises subtypes A1 and A2, and type B, subtype B1. The types differ in their antigen composition and can easily be differentiated in experiments with animal or human convalescent sera. Serum of a patient who has recovered from virus A influenza inactivates and neutralizes type A but has no effect on type B.

The influenza virus contains two antigens: a soluble antigen which

is associated with the virus nucleoproteid and a haemagglutinin contained in the viral protein membrane, which possesses enzymatic properties owing to the presence of mucinase. The soluble antigen is common to all types and subtypes while the haemagglutinins are characterized by type and subtype specificity. The common antigen is demonstrated best by the complement-fixation reaction and the haemagglutinin by the haemagglutination-inhibition test.

According to their behaviour in the haemagglutination-inhibition test, three strains of the influenza A2 virus are distinguished: (1) nonavid strains whose haemagglutinins are neutralized only by immune type A2 sera; (2) avid strains whose haemagglutinins are neutralized by immune type A, A1, and A2 sera; (3) polyavid strains whose haemagglutinins are neutralized in the haemagglutination-inhibition test with immune type A, A1, A2, and B sera.

An extremely characteristic property of the influenza virus is its ability to display wide variability during epidemic outbreaks. Studies of this problem have shown that strains isolated at the onset of the outbreak differ from those recovered at the end.

The influenza virus has been proven to change in antigenic structure within a short period of time. Influenza virus A1 did not exist prior to 1945-47, but developed as a result of variations in the antigenic properties of virus A. As is known, the world-wide influenza pandemic of 1957 was caused by a new subtype of influenza virus A2 (Asia 57).

Variations in the chemical composition of the virus body concern only the haemagglutinin antigen and not the soluble antigen. Immunity produced by influenza is both species-specific and type-specific; this property of the influenza virus is taken into account when carrying out specific therapy and prophylaxis and in laboratory diagnosis.

Resistance. The virus remains viable in a dried state and at low temperatures for quite long periods. It withstands exposure to glycerin, in which it retains its activity for 3 months. The influenza virus is of low resistance and is killed at 65°C in 5-10 minutes. It is very sensitive to drying and is rapidly destroyed in both alkaline and acid media. It is readily inactivated by all disinfectants: chlorinated lime, chloramine, formalin, etc. Ultraviolet irradiation and supersonic vibration are destructive to the virus.

Pathogenicity for animals. Pigs, horses, and some birds are naturally susceptible to certain species of the influenza virus. Type A and B viruses readily adapt themselves to many species of domestic animals during epidemics but have no epidemiological significance for human beings. Among the laboratory animals most susceptible are African ferrets. Two days following inoculation there is an elevation of temperature, catarrhal involvement of the upper respiratory tract, nasal discharge of a purulent character, and sneezing. Mice and young pigs are also susceptible to influenza.

Pathogenesis and disease in man. Having entered the body of a susceptible individual by the nasopharynx, the virus penetrates the cells of the surface epithelial layer of the upper respiratory tract mucosa.

The influenza virus is strictly pneumotropic and multiplies only in the cells of the respiratory tract epithelium. As the virus multiplies and the infection develops, the trachea, bronchi, bronchioli, and alveolar epithelial cells gradually become involved in the process. The injured ciliated epithelium loses its cilia and as a result of this becomes deprived of its defence function. It serves as a favourable medium for the penetration of secondary bacterial flora (streptococci, pneumococci, *H. influenzae*, etc.) which give rise to complications (bronchitis, pneumonia, pleuritis, encephalitis, influenzal meningitis, and ditis media). In addition, influenza activates chronic conditions (tuberculosis) and significantly lowers immunity to a number of infections.

Immunity is associated with the presence of virucidal and virus-neutralizing antibodies. The condition of the throat is also of importance. The labile antibodies are the most active. They are produced relatively quickly in high titres in influenza patients. Interferon which is capable of inhibiting the multiplication of influenza virus plays a definite role in conferring immunity to influenza. It is also present in a healthy body, but in small number. The number of interferons greatly increases in the presence of the influenza virus. Reproduction of artificial immunity with live vaccine gives rise not only to accumulation of antibodies but also to a block of the sensitive cells and also to interference with the natural influenza virus.

Laboratory diagnosis. The haemagglutination reaction is the most simple and available of all the methods employed for laboratory diagnosis of epidemic influenza. It is based on the ability of the erythrocytes (guinea pig, hen, dog, human 0 blood type) to adsorb the influenza virus on their surface and thus become agglutinated. This phenomenon is due to the presence of an enzyme, haemagglutinin, in the influenza virus. Each virus type possesses a specific haemagglutinin. When the virus is treated with specific serum, the haemagglutinin is neutralized by antibodies, i.e., antihaemagglutinins.

The mechanism of the reaction is based on the fact that the influenza virus haemagglutinin reacts with the erythrocyte receptors. On completion of the reaction the haemoglobin is liberated and reacts again with new erythrocytes.

The haemagglutination reaction is used for influenza diagnosis. It reveals the presence of both the virus and the antibodies.

There are various modifications of the haemagglutination reaction (i.e., carried out on glass. in test tubes, or on glass slides which have depressions). The test material is collected from the patient's throat-

gargling washings on the first-third day of the disease. The washings are cleared by centrifugation or filtration through cotton wool.

The virus type is determined by the haemagglutination-inhibition test using type virus-neutralizing sera. The virus loses its agglutination ability when neutralized by the serum.

As the haemagglutination reaction with throat washings is not strictly specific, it is seldom used nowadays. For demonstrating the influenza virus, 11-12-day-old chick embryos are inoculated with patient's nasopharyngeal washings in special laboratories. After 3 days of cultivation the virus is detected by the haemagglutination reaction or by complement-fixation reaction with allantoic or amniotic fluids. A number of passages are made for isolating the virus and adapting it to growth in a developing embryo.

The influenza virus grows readily in one-layer trypsin-treated tissues prepared from kidneys, monkey heart, etc.

These tissues are infected with patients' mouth washings. After 2-3 days of incubation, the virus is detected by the adsorption reaction. This reaction is performed in the following manner: 0.2 ml of a 4 per cent suspension of sterile guinea pig or hen erythrocytes is transferred into test tubes containing monkey kidney tissue in which the influenza virus is being grown. The test tubes are left in a tilted position for 3-5 minutes at room temperature and then placed into a roller drum for several minutes to remove the unadsorbed erythrocytes from the cell monolayer.

The cell layer is examined under the microscope at low magnification. The reaction is considered negative if the erythrocytes are observed floating in the field of vision without being adsorbed on the monolayer or if only single erythrocytes are adsorbed. A positive reaction is indicated when erythrocytes can be seen adsorbed on the monolayer in clusters, rosettes, chains, and bands.

In addition to these relatively early diagnostic methods, retrospective serologic methods (complement-fixation, haemagglutination-inhibition, and neutralization reactions) are used. For these reactions the blood is collected during the first days of the disease or during the recovery period. Paired sera are examined for a rise of antibody titre which is usually fourfold in convalescents.

Rhinocytoscopy is a less specific method but is convenient for general use. Imprints of the mucosa of the inferior nasal conchae, made on narrow polished glass slides, display conglomerations of columnar epithelium cells and specific intracellular inclusions in 60-90 per cent of influenza cases.

Virusoscopy of smears impregnated with silver according to Morozov is employed as an additional method. However, the disadvantage of this method is that it does not allow for differentiation of the influenza virus from viruses of other diseases.

Treatment. Dry or liquid sera are used for treatment and prophylaxis. They are introduced intranasally 2-3 times a day.

The sera are prepared by horse hyperimmunization with strains of the more widely occurring virus types and from the blood of human beings who have suffered from influenza.

The dry anti-influenzal (horse) sera contain sulphonamides (norsulphazol, etc.) which prevent the development of various complications caused by bacterial microflora.

Complications are prevented by antibiotics (penicillin, chlortetracycline, etc.) given in association with sulphonamides. One would expect interferon to be effective in the treatment of influenza patients.

Prophylaxis. Influenza is spread by the air-droplet route. The source of infection is an influenza patient who may infect healthy people when sneezing, coughing, and talking.

Influenza is highly contagious. It causes epidemics and pandemics which occur at definite intervals (see p. 197).

Spread of influenza is prevented by isolating the patients, regularly ventilating the rooms and cleaning them with a damp cloth (moistened in chloramine solution). Physical culture and sports serve to strengthen the body.

Human beings are vaccinated against influenza with a vaccine prepared from various strains of given types of influenza virus. The viruses are cultivated in chick embryos. The cultures are examined for sterility, innocuousness, and immunogenicity. Liquid and dry vaccines are available. The vaccine is introduced either into the nose in drops or into the upper respiratory tract with a pulverizer.

At present dry anti-influenzal vaccines are produced (monovalent vaccine containing one virus type and polyvalent vaccine containing two or three virus types). However, preparation of an anti-influenzal vaccine is still a matter of difficulty owing to extremely rapid changes taking place in the antigenic structure of the influenza virus.

PARAINFLUENZA VIRUSES

The myxovirus group includes the aetiological agents of parainfluenzal infections, which have been isolated in various countries from cases with respiratory tract lesions.

Morphology. The viruses range from 110 to 150 mμ in size. There is a double nucleoproteid spiral measuring 18 mμ within the virions, and it is twice as large as that of the influenza virus. Under the electron microscope the parainfluenza viruses are seen to be spherical in shape.

Cultivation. The parainfluenza viruses are incapable of multiplying in chick embryos, but they grow readily in tissue cultures from monkey kidney epithelium and human embryos and on human embryo fibroblasts. Some types can be adapted to subinoculated Hep-2, HeLa, and other tissue cultures.

Antigenic structure. Parainfluenza viruses are divided into 4 types: type 1 (Sendai virus) is responsible for croup in children, pharyngitis, bronchiolitis, and pneumonia; type 2 produces laryngotracheobronchitis and catarrhal diseases of the upper respiratory tract; types 3 and 4 have been recovered from children suffering from croup and lung lesions. The viruses agglutinate guinea pig, hen, and human erythrocytes.

Resistance. Parainfluenza viruses are sensitive to ether. When frozen they remain viable for 1-2 months. They are killed relatively quickly by heating and exposure to the action of disinfectants.

Pathogenicity for animals. Fatal isolated outbreaks as well as large epizootics occur among cattle. In laboratory conditions, guinea pigs and Syrian hamsters are susceptible to the organisms.

Pathogenesis and diseases in man. Parainfluenza viruses type 1, 2, and 3 are more frequently recovered from children suffering from upper respiratory tract lesions such as rhinitis, pharyngitis, and bronchitis, which are accompanied by a rise of temperature. Parainfluenza virus type 2 is responsible for croup. It has been isolated in 26-51 per cent of cases. Antibodies for the type 2 virus have been demonstrated in 35-57 per cent of patients.

It is very seldom that parainfluenza viruses are the cause of severe diseases in adults, and usually they produce mild diseases of the respiratory tract. Quite frequently, parainfluenza viruses occur in association with the influenza virus and with adenoviruses.

Laboratory diagnosis. Differential diagnosis of influenza and parainfluenza infections presents great difficulties. It is based mainly on virological and serological examinations. Monolayer cultures from monkey kidneys or human embryos are used for isolating the virus. The presence of the virus in tissue cultures is demonstrated by haemadsorption. The isolated strains are identified by the neutralization reaction, haemadsorption-inhibition reaction on tissue cultures, haemagglutination-inhibition test, and complement-fixation reaction.

Acute respiratory infections may be caused by the respiratory syncytial virus RS. It was first isolated in the USA from chimpanzees and later from children with respiratory diseases. It was designated RS owing to the fact that virus multiplication causes syncytium formation (a netted tissue the cells of which are connected by cytoplasmic processes). The virus is 90-120 mμ in size and contains RNA. It is sensitive to ether and freezing and is inactivated at 56°C in 30-60 minutes. The RS virus does not multiply in chick embryos. It is nonpathogenic for laboratory animals. No haemagglutinins are produced in tissue cultures, and no haemadsorption occurs. It grows in Chang, Hep-2 and HeLa subinoculated human cells. In human beings it is responsible for catarrhal conditions of the upper respiratory tract, bronchitis, bronchiolitis, and pneumonia. Infections occur as epidemic outbreaks of 2-3 months' duration. They are

quite frequent in children during the winter to spring season. Occurring among groups of children the infections affect almost every child. RS virus epidemics recur annually, as distinct from parainfluenza infections which occur as endemics several times a year. As has been ascertained in virus studies on volunteers, the incubation period lasts from 4 to 9 days, while the disease is of 5-6 days' duration. The antibody titre in the blood has no effect on the result of infection.

It has been proved recently that other viruses (over 70 in number) may also produce acute respiratory diseases. They include the reoviruses (types 1, 3, and, less frequently, type 2), ECHO viruses (types 8, 11, 20, and 28), rhinoviruses (Hyp, FEB, and others), Coxsackie A viruses (type 21 and, less frequently, types 1, 2, 4, and 5), and Coxsackie B viruses (types 5, 3, 2 and, less frequently, types 4, 1, and others).

EPIDEMIC PAROTITIS (MUMPS) VIRUS

The causative agent was discovered in 1934 by C. Johnson and E. Goodpasture.

Morphology. As can be seen under the electron microscope, the virus has a dome-like elongated irregular shape. It is from 150 to 233 mμ in size and can be stained readily by Morozov's method.

Cultivation. The virus multiplies in developing chick embryos. Freshly isolated strains grow well in monkey kidney epithelium, HeLa tissue cultures, etc.

Antigenic structure. The virus of epidemic parotitis possesses no serologic types. It contains two antigens: viral and soluble. The viral antigen is larger, infectious, is adsorbed on erythrocytes and causes their agglutination. It is found in chick embryo fluids. The soluble antigen is considerably smaller, devoid of infectious properties, is not adsorbed on erythrocytes and does not cause agglutination of the latter. It occurs mainly in chick embryo membranes.

The virus of epidemic parotitis can cause haemagglutination and haemolysis of human, sheep, hen, etc., erythrocytes. Its haemagglutinins and haemolysins are neutralized by specific antibodies and by nonspecific inhibitors contained in the serum and various biological substrates.

Resistance. The virus survives at low temperatures (—25 and —70°C) up to 10 months. It is weakly resistant to exposure to physical and chemical factors. A temperature of 55-60°C kills the virus in 20 minutes. It is inactivated quickly by ultraviolet radiation and destroyed by 0.1 per cent formalin, 1 per cent lysol, 50 per cent alcohol, or ether.

Pathogenicity for animals. The virus of epidemic parotitis does not naturally produce disease among animals. Experimental infection can be produced in some monkey species, the resulting clinical

picture being similar to that in human parotitis. The virus can be adapted to some animals (hamsters, white mice, white rats) by intracerebral inoculation of one-day-old sucklings. Inoculation is followed by severe encephalitis terminating in death.

Pathogenesis and disease in man. The parotitis virus causes fever and inflammation of the parotid, sublingual, and submandibular glands. The disease is prevalent among children. Parotitis is a very contagious disease. Severe cases are accompanied by virusaemia. In addition to causing lesions in the salivary glands, the virus penetrates other organs and produces orchitis and meningitis. Complications include polyneuritis, paresis of the facial and auricular nerves, and functional disturbances of the organs of vision and hearing.

Immunity. A high-grade and, practically, lifelong immunity is produced following the disease. Complement-fixing and haemagglutinin-inhibiting antibodies appear in the serum.

Laboratory diagnosis is made by serological examination, i.e., the complement-fixation reaction and haemagglutination-inhibition test. Antibodies may be detected a week after the onset of the disease, their titre rising intensively. Examination of paired sera yields the best result.

Treatment. Patients with epidemic parotitis are prescribed symptomatic drugs: e.g., corticosteroids and gamma-globulin. These drugs reduce the severity of the disease but do not prevent the development of meningitis and orchitis.

Prophylaxis. General measures comprise isolation of patients until they recover. All child contacts younger than 10 years of age are isolated for a period of 21 days (the longest incubation period). Patients are discharged from the hospital only when all clinical manifestations of the disease have disappeared. No disinfection is carried out. Specific prophylaxis is accomplished with injections of gamma-globulin and vaccination with a live vaccine obtained by A. Smorodintseff and N. Klyachko. The live vaccine is prepared from epidemic parotitis virus strains which have lost their pathogenicity as a result of passage on chick embryos. It is introduced intracutaneously in a single dose.

THE MEASLES VIRUS

The viral nature of measles was proved in 1911 by J. Anderson and J. Goldberger. The aetiological agent was grown by Plotz in 1938.

Morphology. The virus is from 90 to 100 mμ in size.

Cultivation. The virus is cultivated on various tissues and on monolayer cultures of human, monkey, and dog kidney epithelium. A cytopathogenic effect has been obtained on human amnion cell cultures.

Antigenic structure and classification. No varieties or types of the measles virus strains have been recognized. Vaccine strains identical in morphological properties to the natural strains have been obtained. Specific prophylaxis is based on this peculiarity. Human beings who have recovered from measles are capable of producing antibodies against the virus for a long period of time.

Resistance. The causative agent is very susceptible to high temperatures and is destroyed quickly at 58°C. Outside the body it survives for not longer than 30 minutes. It is extremely sensitive to exposure to sunlight. Because of these properties, disinfection is not carried out in measles.

Pathogenicity for animals. Monkeys are slightly susceptible to this virus. In nature the measles virus is parasitic only in the cells of the upper respiratory tract of human beings.

Pathogenesis and disease in man. Man is the only source of infection. He becomes infective from the first day of the prodromal period and remains so until the fourth or fifth day after the appearance of eruption. The total duration of the contagious period is 8-10 days. Measles is spread by the air-droplet route. The disease prevails in winter. Overcrowding contributes to an increase incidence of the disease.

Measles causes an anergic condition and lowers immunity to influenza, tuberculosis, diphtheria, whooping cough, scarlet fever, and other infections. As a result of changes in the immunological reactivity, measles is quite frequently accompanied by widely varying complications caused by the measles virus itself and by secondary bacterial flora. After entering the upper respiratory tract, the virus invades the blood and affects the tissues of the respiratory tract. The disease is accompanied by virusaemia, fever, and eruption. Infection prevails in children. Measles may attack adults who have not contracted the disease previously. For example, there were no cases of measles for a period of 49 years in the Kolyma Range. It was introduced there in 1901, and all the inhabitants were affected by the disease as a result of which about 7 per cent of them died. There was a measles epidemic in the Fiji Islands in 1875 when 150,000 people contracted the disease and 40,000 of them died. Many times the disease was introduced to the Faeroe Islands (1781, 1846, 1862, 1875). An epidemic was recorded in southern Greenland in 1951 and in northern Canada in 1952. All these facts provide evidence of the extremely high infectiousness of measles in human beings of all ages. Children who have been given prophylactic injections of antimeasles serum or gamma-globulin acquire a mild (mitigated) form of the disease.

Immunity. Measles produces high-grade immunity of long duration which is humoral in character. Re-infections hardly ever occur. Individuals who have had measles harbour antibodies in their blood for life.

Laboratory diagnosis. Measles is identified on the basis of clinical and epidemiological data. Laboratory examination methods are not widely used at present. V. Ioffe proposed the complement-fixation reaction. P. Sergiyev and collaborators studied agglutination of bacteria which had adsorbed the virus.

Treatment. There is no specific treatment. In cases of complications antibiotics are prescribed (penicillin, chlortetracycline, streptomycin).

Prophylaxis. Sick children are not normally sent to the hospital, but are isolated at home. Only those living in hostels or suffering from severe forms of the disease are transferred to hospital.

Children who have been in the vicinity of a measles tocus and have not been given gamma-globulin are isolated for 17 days, while those who have received gamma-globulin are isolated for 21 days. If the patient has not been isolated, segregation of healthy child contacts is prolonged for a further 5 days.

Rooms which have been occupied by a patient with measles must be ventilated and be looked after in adequately hygienic conditions.

At present, gamma-globulin in 1.5 or 3 ml doses is employed for measles seroprophylaxis. It is prepared from donor or placental serum. Gamma-globulin renders no effect if it is introduced after the seventh day of the incubation period.

Passive immunity persists for 30 days. If a child was exposed to contact with a measles patient for a second time, the gamma-globulin injection is repeated.

Usually gamma-globulin does not completely provide protection from the disease, but delays its onset, significantly lessens its severety, and prevents fatal cases.

Intensive research work is carried out in the field of anti-measles vaccine study in special institutes in the USA and USSR. Smorodintseff's live anti-measles vaccine is being tested on a wide scale and with good results. It is injected subcutaneously in a single dose. Its reactivity-producing ability is reduced by injecting gamma-globulin simultaneously.

EPIDEMIC POLIOMYELITIS VIRUS

In spite of the fact that poliomyelitis is one of the most ancient contagious diseases, its infectious nature was ascertained only in 1905 by O. Wickman who investigated a poliomyelitis epidemic in Sweden.

In 1908-09, K. Landsteiner and E. Popper proved poliomyelitis to be of viral aetiology. They produced a febrile disease in monkeys by injecting an emulsion prepared from the spinal cord of a fatal case of poliomyelitis. The animals displayed typical manifestations of poliomyelitis accompanied with flaccid paralysis. The virus was isolated in tissue culture in 1949 by J. Enders.

Morphology. The virus (Fig. 134) ranges from 8 to 27 mμ in size and forms intranuclear inclusions. The poliomyelitis virus has been obtained in crystalline form. The organism is devoid of fermentative systems, it is fully dependent on host cells and is an obligatory intracellular parasite. Its contagious properties are associated with nucleic acid.

Cultivation. The virus multiplies on a medium consisting of Tyrode's solution, monkey serum, and small pieces of the brain of a 10-12-day-old chicken embryo, or on tissue cultures of monkey kidneys, human embryo or HeLa cells. Spherical bodies which are 0.2 μ in size and stain light-blue or violet with the Romanowsky-Giemsa stain are found in the cells of tissue cultures.

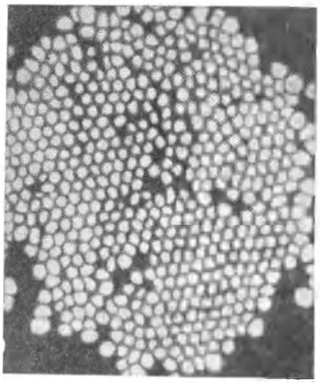


Fig. 134 Poliomyelitis virus

Antigenic structure. There are three types of viruses which do not possess common immunogenic properties.

Type I viruses include the Brunhilde strain (USA) and the KPΦ strain (USSR), which are pathogenic for human beings and monkeys. They are identical as regards immunological properties. Type II viruses include the Lansing strain (USA) and the Ovchinnikov strain (USSR), which are responsible for diseases not only in human beings and monkeys, but also in rodents (cotton rats, white and grey mice, field voles, hamsters, etc.). They do not produce immunity to strains of other types of poliomyelitis virus. Type III viruses (the Leon strain in the USA and the Zonders strain in the USSR) are pathogenic only for human beings and monkeys and differ from the first two types in their immunological properties.

During epidemic outbreaks, type I is most frequently isolated (in 65-95 per cent of cases) while types II and III account for the remaining 5-35 per cent of cases.

Viruses identical to the poliomyelitis virus and Coxsackie virus were isolated in 1952-59. Paralytic forms of the disease are more frequently produced by the type I organism. It causes production of virus-neutralizing and complement-fixing antibodies in human and animal bodies.

Resistance. The virus is extremely resistant to photodynamic inactivation. It survives in sterile water at room temperature for a period of more than 100 days, in milk for 90 days, in faeces in the cold for more than 6 months, and in sewage for several months. It withstands exposure to 0.5-1 per cent phenol solutions and remains viable for several weeks at pH 3.8-8.5.

The poliomyelitis virus is very sensitive to calcium chlorate lime, chloramine, formalin, potassium permanganate, and hydrogen peroxide solutions. It is rapidly killed on boiling.

Pathogenicity for animals. Enteroviruses of monkeys, cattle, swine, and other animals were found to be similar to poliomyelitis and ECHO viruses. However, their connection with the poliomyelitis virus has not been ascertained. Chimpanzees and rhesus monkeys are easily infected with type I and III strains in experiments, and cotton rats and white mice are susceptible to the type II virus. Disease is produced on the third-eighth day following inoculation, with manifestation of flaccid paralysis of the back and extremities.

Pathogenesis and disease in man. The virus is extremely neurotropic. It causes development of degenerative inflammation processes in the anterior horns of the spinal cord and subcortical grey substance. However, the virus circulates in the blood and is discharged through the nasopharyngeal mucous membranes. It may be discharged with the faeces of patients and convalescents for 2-7 weeks and, sometimes, up to 4 months, as well as during the incubation period. Healthy carriers also excrete the virus with the faeces. The poliomyelitis virus may be found in nasopharyngeal mucus and tonsils approximately 3 days before the temperature rises and for 3-10 days after the onset of the disease. According to the clinical symptoms, three forms of poliomyelitis are distinguished: abortive, nonparalytic, and paralytic.

Immunity. A very stable immunity is produced following the disease. No cases of re-infections have been described. Immunity is associated with the presence of virus-neutralizing antibodies in the blood.

Laboratory diagnosis is made by performing the neutralization test and the complement-fixation reaction. The latter is similar in its mechanism to reactions employed for diagnosing bacterial and rickettsial diseases. The antigens are prepared from a brain suspension of mice infected with a particular virus strain, from tissues of chicken embryos inoculated with a virus, and from tissue cultures of other origin. Antigens are subjected to special treatment for removal of their anticomplement properties. The antigens must be clear and should be stored in a frozen state. They are preserved by adding merthiolate in a ratio of 1 : 10,000.

The complement-fixation reaction may be used for detecting both antigens and antibodies in the body of sick human beings and animals. Sera for antigen detection are obtained from immune animals. Homologous sera are used for antigen examination, this condition being disregarded for convalescent sera.

The complement-fixation reaction is based on the same principles as serological diagnosis of bacterial infections. The main test involves test and control antigens, test and control sera, the complement, and the haemolytic system.

This reaction reveals the presence of the antigen (virus) in the body and its increase in number in patients with manifest and latent forms of the disease. Antibodies to a definite antigen are found in convalescents. The complement-fixation reaction methods are improved upon every year: fixation reaction in the cold, micromethod, and others.

The neutralization test demonstrates that the virus can be rendered harmless by specific immune sera. It is widely used for recognition of many viral infections. Its mechanism is not clear to this day. Some authors maintain that it is analogous with the reaction between an antitoxin and exotoxin. Others describe it as the result of antibodies of the antiviral sera blocking the susceptible cells.

The neutralization test may be performed by various methods. Two components take part in the reaction, the serum and the virus. Whole serum is mixed with equal volumes of increasing dilutions of the virus, or a certain dose of virus is mixed with various serum dilutions. The mixture is inoculated into animals (subcutaneously, intraperitoneally, or intravenously, depending on what method of infection will produce the disease).

Checking the results of the neutralization test is a rather complicated procedure. It is necessary to determine the neutralization index (the ratio of the dose of virus mixed with immune serum, lethal to 50 per cent of mice, to the dose of virus mixed with normal serum, causing equal lethality).

According to the USA Committee for Standardization of Serologic Methods, a neutralization index of from 1 to 10 is valued negative, of from 10 to 50 questionable, and of 50 and higher, positive.

The tissue culture method is also valuable. The poliomyelitis virus is grown in cultures of human embryos and HeLa cells. Better results have been obtained with monolayer cultures of testicular and kidney cells on which plates (site of degenerate cells) can be distinctly seen. The presence of the virus may also be revealed by the neutralization test, using tissue cultures. This method helps to make a quick diagnosis and to determine the type of virus.

Complement-fixing antibodies appear in the blood of patients with acute poliomyelitis. Not only the disease, but the type of virus is recognized by their presence.

A relatively quick diagnosis of poliomyelitis can be made with the help of a colour test. Various dilutions of the virus and, simultaneously, phenol red as indicator are introduced into test tubes which contain a tissue culture from monkey kidneys or HeLa cell cultures. The red medium turns yellow in test tubes which contain normal tissues (no virus present) and remains red in those which contain tissues infected with the virus. There are several modifications of the colour test.

Treatment. There is no specific treatment. Early injections of gamma-globulin, blood transfusion, wide use of vitamins C and

B_{12}, amino acids (leucine, glutamic acid), analgesics (analgine, amidopyrine, pantopon, etc.), mediators, and stimulants (proserine, galanthamine, dibazol, etc.) are recommended. An orthopaedic regime is set up from the first day that paralysis develops, for prevention of contractures and deformations, and exercise therapy is carried out during the rehabilitation period. An apparatus for artificial respiration is employed when there are respiration disturbances.

Prophylaxis. The sources of infection are human beings suffering from poliomyelitis (with clinically manifest, latent, and atypical forms) and carriers who have been in close contact with the patients. It is considered that only 10 per cent of infected individuals contract the disease, and the paralytic form develops only in 0.1-1 per cent of cases.

The abortive, latent, and asymptomatic forms of poliomyelitis occur most frequently among individuals closely associated with the patient and are dangerous unrecognizable sources of the disease.

Poliomyelitis is transmitted from a patient or carrier by dirty hands, contaminated foodstuffs and water, certain objects used for nursing patients, underclothes and bed-clothes, and by flies, particularly in summer and autumn. Air-droplet transmission is also possible. The incidence of the disease prevails in the summer and autumn months (August-September), but single cases occur throughout the year.

Poliomyelitis is prevalent among children from 4 months to 5 years of age. Cases occur among older children and adults during epidemic outbreaks of the disease.

Prophylactic measures comprise early, appropriate diagnosis and active recognition of the disease in the out-patient department and during house calls as well as when examining children in children's institutions and hospitals, etc. All patients with acute forms of poliomyelitis and those suspected of having the disease must be instantly transferred to a hospital. Milk intended for consumption by children must be pasteurized. Water is rendered harmless by boiling or chlorination. Poliomyelitis contacts are not admitted to children's institutions for 20 days.

Active vaccination is accomplished with vaccines prepared from a virus inactivated with formalin (J. Salk) or from attenuated virus strains (A. Sabin). In the USSR, A. Smorodintseff and M. Chumakov have worked out a method of mass vaccination of children with a live vaccine. As a result of such vaccination, poliomyelitis disease rate has decreased significantly. Vaccination with live vaccine helps to solve successfully the problem of complete elimination of poliomyelitis.

The inactivated polyvalent vaccine consists of type I, II, and III poliomyelitis viruses killed with formalin. It is administered intramuscularly (into the upper third of the left arm) in 3 doses. The live vaccine is produced in the form of small sweets which con-

tain a definite type of the poliomyelitis virus. It is given orally in 3 doses.

Specific passive immunization is accomplished by injecting gamma-globulin, parent's blood, or sera of healthy individuals into all children under 7 years of age who have been in contact with poliomyelitis patients. Older children are given injections according to medical indications. Passive immunity lasts for three weeks.

COXSACKIE AND ECHO VIRUSES

Diseases caused by the Coxsackie virus were discovered by G. Dalldorf and G. Sickles in 1948 in Coxsackie (New York State) among children suffering from paralysis and other infections resembling poliomyelitis. The virus, isolated from two child patients, proved to be pathogenic to newborn mice, producing fatal diseases with paralysis and destructive lesions of the striated muscles. Later, Coxsackie viruses were found in many countries of the world. The ECHO viruses (enteric cytopathogenic human orphans) were discovered by J. Melnick, J. Enders, et al.

Morphology. The viruses are extremely small, being 25-30 mμ in size, and pass through bacterial filters.

Cultivation. The viruses are grown on tissue cultures (human and monkey kidney cells, human embryo fibroblasts). The majority of ECHO viruses are nonpathogenic for newborn mice.

Antigenic structure. There are 30 types of the Coxsackie virus which differ in immunological properties. Their presence was revealed by experiments with cross neutralization of antibodies and by the complement-fixation reaction.

Two subgroups of the Coxsackie virus are distinguished: A (24 types) and B (6 types). This group of viruses possesses immunogenic properties. Data on interference of Coxsackie viruses with poliomyelitis viruses are contradictory. It is probable that in relation to immunogenic properties this group is heterogenous.

There are 28 types of ECHO viruses which are responsible for diseases with a numerous variety of clinical manifestations.

Resistance. Coxsackie and ECHO viruses possess relatively high resistance. They survive for a long period of time in a frozen state at —70°C. In glycerin and horse serum at room temperature they persist for 70 days. In a refrigerator they survive for more than a year.

The Coxsackie viruses resemble the poliomyelitis viruses in that they are resistant to various concentrations of hydrogen ions. They survive at pH 2.3-9.4 for 24 hours and at pH 4.0-8.0 for 7 days. A temperature of 50-55°C kills the viruses in 30 minutes. They are resistant to antibiotics, 70° ethyl alcohol, and 5 per cent lysol solutions but are extremely sensitive to solutions of hydrochloric acid and formaldehyde.

Pathogenicity for animals. It has been recently proven that cattle, birds, young swine, and rodents contract viral enteric diseases. It is probable that in some cases they are sources of human infection.

Pathogenesis and diseases in man. The clinical picture is variable. There are several clinical forms of the disease (Table 32).

Table 32

Diseases Produced by Enteroviruses

Virus	Disease
Poliomyelitis	Poliomyelitis, aseptic meningitis, undifferentiated febrile diseases
Coxsackie, A subgroup (24 types)	Herpangina (types 2, 3, 4, 5, 6, 8, and 10) Febrile diseases, accompanied by petechial and vesicular rash and stomatitis (types 9 and 16) Aseptic meningitis (types 4, 7, 9, 23), epidemic forms Respiratory infections (type 21) Paralytic forms similar to poliomyelitis (types 4, 7, 9, 23) Pericarditis (type 1)
Coxsackie, B subgroup (6 types)	Pleurodynia (Bornholm disease) (types 1, 3, 5) Aseptic child myocarditis (types 3, 4) Aseptic meningitis (all six types) Vesicular pharyngitis (types 2, 6) Febrile diseases with exanthema (types 1, 3, 5) Paralytic diseases similar to poliomyelitis (types 3, 4, 5) Hepatitis in pregnancy (type 5) Parkinson's syndrome (type 2) Encephalitis (type 3)
ECHO (28 types)	Aseptic meningitis (17 types) Respiratory infections (types 8, 11, 20, 22, 25, 28) Enterovirus diarrhoea (types 6, 14, 18, and others) Cerebral ataxia (type 9) Paralytic diseases similar to poliomyelitis (types 2, 4, 9, 11, 13, 16) Febrile diseases accompanied by rash (types 9, 16)

Aseptic serous meningitis. The disease is accompanied by an elevation of temperature, indisposition, severe headache, nausea, and stomach pains. These symptoms are later followed by rigidity of the neck muscles. One or two days later vomiting occurs, and the temperature rises to 40°C and higher. The fever continues for 5 days (the duration may vary from 3 to 10 days). Relapses of fever with 4-5-day intervals occur. Hyperaemia of the throat is noted in some cases. Examination of spinal fluid reveals leucocytosis of 200 cells per ml (sometimes 600-800 cells per ml). The disease lasts from 1 to 3 weeks.

Pleurodynia (Bornholm disease). The infection is characterized by fever, rise of temperature to 38-40°C, headache, pain in the throat on swallowing, pain in the muscles, chest, abdomen, and extremities. The pains intensify whenever the patient moves, and last

from 2 days to 2 weeks. The fever lasts for 2-4 days, and a relapse of 2-3 days duration may sometimes occur. In 50 per cent of the cases, the pain is localized in the abdomen, but it may occur in the trunk or extremities. In some patients rigidity of the neck or back appears. This disease is identical with aseptic meningitis as regards the duration of fever.

Herpangina. The disease is characterized by a sudden onset of fever (up to 40.5°C) of 1-4 days duration, loss of appetite, and swallowing difficulties. The majority of patients complain of a sore throat, and vomiting and abdominal pain occur in 25 per cent of cases. The throat is hyperaemized and covered with clearly defined papules localized in the anterior folds of the fauces and, less frequently, on the palate, uvula, tonsils, and tongue. At first the papules (from 5 to 14 in number) are greyish-white and surrounded by a red circle. Later, the redness of the circles deepens, the papules become enlarged and then ulcerated. The vesicular and ulcerated lesions sometimes develop simultaneously. Leucocytosis develops in the blood.

Boston (epidemic) exanthema. The disease is accompanied with fever lasting 3-4 days, chills, severe headache in adults and abdominal pains in children, skin eruptions resembling those in rubella, and development of vesicles and ulcers in the throat. It is an epidemic disease.

Aseptic myocarditis. Occurs among newborns and up to 3-year-old children. It is characterized by a very high death rate (from 70 to 80 per cent).

Outbreaks of encephalomyelitis of newborn children may occur in maternity hospitals. This disease becomes severe and has a high death rate. Enterovirus diarrhoea may also be encountered, it occurs in early childhood.

The Coxsackie and ECHO viruses may be responsible for a number of other diseases: nondiphtheritic croup, influenza-like and poliomyelitis-like diseases.

Three-day fever. This disease begins with an elevation of temperature to 38-39°C and is accompanied with a sore throat, pains in the abdomen and epigastrium, hyperaemia of the face and throat, and injection of the sclerae and conjunctiva.

Immunity. The disease leaves quite a high-grade immunity. In addition, immunity is produced as a result of latent forms of enteroviral infections, particularly among elder groups of the population. The supposition that the Coxsackie virus and the aetiological agents of other diseases of this group can be transmitted by the air-droplet route calls for research of methods of specific enterovirus prophylaxis. Vaccines prepared from killed and live viruses are at present being tested.

Laboratory diagnosis. The disease is recognized by its clinical manifestations and epidemiological and laboratory data.

Virological investigations comprise examination of nasopharyngeal and tonsillar excretions, spinal fluid, and faeces. The material under test is inoculated into 2-day-old cotton rats and mice. Inoculation is followed by the development of characteristic changes in the sceletal muscles, focal lesions in the brain, muscles, and other organs.

The virus is recovered from faeces and nasopharyngeal washings of the patients. It is very pathogenic for suckling mice and hamsters who display lassitude and paralysis of one or several extremities and of the neck. Death occurs within 24 hours following inoculation. Diagnosis is also made by employing the virus neutralization reaction and by the rising titre of antibodies in convalescents as compared to that in the initial stage of the disease. Viruses of group A affect the striated muscles of suckling mice and produce generalized myositis. Viruses of group B are responsible for paralysis and pathological changes in the internal organs of animals.

Differentiation of Coxsackie virus infections from poliomyelitis is of great practical importance as in some cases the time of commencement of these diseases coincides.

Treatment has not yet been worked out. At present, there are no specific drugs affecting the Coxsackie viruses.

Prophylaxis. Viruses of the Coxsackie and ECHO group are widespread. Patients discharge them with the faeces and from the nasopharynx. They are recovered from sewage and may be transmitted by flies. Disease incidence prevails in summer and early autumn. The existence of Coxsackie virus carriers has been proven. The disease may be contracted by human beings of any age and sex, but prevails among children. There are no specific prophylactic methods. General sanitary measures are carried out, similar to those employed in cases of intestinal infections although the possibility of the air-droplet route of transmission is not excluded. As in cases of intestinal and respiratory infections, isolation of patients, disinfection, and other measures are carried out.

Many other viruses have been discovered within the last ten years. They have been isolated from intestines of human beings, particularly of children in whom they produced various clinical symptoms. Viruses have been recovered from absolutely healthy human beings, and some types have been isolated from animals. Three types of reoviruses (respiratory-enteric viruses) can be distinguished among the enteroviruses. They range from 60 to 90 mμ in size, contain RNA, and form cytoplasmatic inclusions. They are resistant to ether and heating to 56°C.

A characteristic feature of enteroviruses is that they are not strictly specific. Various types of viruses may produce the very same disease, while viruses of one type may be responsible for quite a variety of infections.

ECHO and Coxsackie viruses and adenoviruses may be the aetio-

logical agents of enteritis in children. The clinical syndrome may be produced by each type acting separately or in association with *E. coli*, dysentery bacteria, and salmonellae. In spite of the fact that the enteric viruses are widespread, they produce diseases only under unfavourable conditions (overheating, infection with bacterial microflora, etc.).

RHINOVIRUSES

The viral nature of contagious rhinitis (common cold) was known more than 50 years ago, but cultivation of the virus became possible only in 1960. Rhinoviruses were isolated from patients suffering from acute rhinitis on primary human kidney cultures, and on monkey kidney subcultures. They are grown at low temperatures (32-33°C) in test tubes shaken for increased aeration and with the pH of the medium adjusted to 6.8-7.2. Strains H and M are known. H-strains multiply only in human kidney cultures, while M-strains, in monkey kidney cultures. The rhinoviruses are identical with the enteroviruses in size, resistance to ether, and in their nonpathogenicity for suckling mice and other animals. In their route of transmission they are similar to the viruses of the upper respiratory tract. In human beings the rhinoviruses occur in the epithelial cells of the nasal mucosa and have not been recovered from the intestines. People with a low antibody blood content are susceptible to infection. Individuals with a high titre of antibodies are immune to rhinovirus infection.

FOOT-AND-MOUTH DISEASE VIRUS

In 1897, F. Loeffler and P. Frosch proved that fluid obtained from foot-and-mouth vesicles remained virulent after passing through bacterial filters.

Morphology. The virus is 8-25 mμ in size and forms eosinophilic intracellular inclusions.

Cultivation. The virus is cultivated in chick embryo cultures to which bovine tongue epithelium tissue has been added. It multiplies quite intensively in the brain tissue of 7-10-day-old mice.

Antigenic structure. The causative agent of foot-and-mouth disease is serologically heterogeneous. Three main types, O, A, and C, and four supplementary types are known. Within the O type six subtypes have been distinguished (O1, O2, O3, O4, O5, and O6).

Resistance. The virus is very resistant to exposure to environmental factors. It survives for 2 months in animal excretions and for up to 2 weeks on the hair. In meat of infected animals it survives for 2-3 days. It is inactivated by disinfectants; e.g., formalin and alkalies.

Pathogenicity for animals. Foot-and-mouth disease is a highly infectious disease of cattle, goats, and swine.

Pathogenesis and disease in man. Human beings may contract the disease from vesicle contents, milk, saliva, or urine. Infection is also carried by fodder and various objects used for cattle management. The virus enters the body via the alimentary tract or through injured mucous membranes and skin. The disease is prevalent among children who drink unboiled milk of infected cows. From the site of primary localization, the virus invades the blood and gives rise to virusaemia and aphthous lesions in the mouth mucosa and skin.

Laboratory diagnosis. For recognition of the disease guinea pigs are given intracutaneous inoculations into the foot pad. In 1 to 4 days following inoculation, the temperature rises and vesicles appear on the pad of the infected foot and on the mouth mucosa. Complement-fixation reaction with paired patients' sera collected at intervals of 4-6 days is also carried out.

Immunity is type-specific and is associated with the presence of virus-neutralizing antibodies.

Treatment. Symptomatic treatment is employed: viz., very light diet and treatment of ulcers with a 4 per cent silver nitrate solution. Suppurative complications are treated with penicillin, and mouth rinsing with rivanol or potassium permanganate solutions is recommended in such cases. Injection of immune serum of convalescent or specific immune serum is relatively quickly followed by recovery.

Prophylaxis is ensured by observing the rules of hygiene (boiling the milk and protection of the face and hands when taking care of animals). A vaccine is employed in veterinary practice for prevention of the disease. The vaccine is injected in a single dose into cattle on farms threatened with the disease.

EPIDEMIC HEPATITIS VIRUS

The works of G. Findlay, F. MacCallum, et al. (1942-1944) have proved the infectious nature of the so-called catarrhal jaundice, a condition described by the Russian clinicist S. Botkin in 1888.

Morphology. The viruses are round-shaped and are 12-18 mμ in size. They form intranuclear inclusions within liver cells and are capable of passing through various filters.

Cultivation. Reliable information on cultivation of the virus in tissues was obtained twenty years after the viral nature of epidemic hepatitis had been ascertained. In 1961 W. Rightsell and his collaborators isolated the virus of epidemic hepatitis on a continuously growing Detroit-6 culture in matrices containing a nutrient medium (85 per cent Iggle's solution and 15 per cent unfiltrated serum of a cow foetus). The authors isolated the virus from serum and plasma collected from epidemic hepatitis patients during the pre-icteric period or during the first 4 or 5 days of jaundice. Twenty-four viral

strains were isolated and successfully passaged through a Detroit-6 culture from 4 to 28 times.

Antigenic structure. The virus is characterized by a complex antigenic structure. It causes the production of virus-neutralizing and complement-fixing antibodies in the human body. The gamma-globulin of human blood neutralizes the virus, and this is extremely important from the practical point of view. Three serotypes have been recognized, two of them being responsible for epidemic hepatitis in volunteers, while the third produced no disease.

Experimental strains, injected into human beings, were isolated from the blood 21-58 days following inoculation. Antibodies appeared in the blood on the fifty-sixth day. Often the volunteers recovered before the appearance of antibodies in the blood serum.

Resistance. The virus is highly resistant. It withstands a temperature of 60°C for 1 hour and is not inactivated in water which has a low chlorine content. It survives for more than a year in glycerin and when desiccated under vacuum in the cold.

Pathogenicity for animals. Animals are insusceptible to the virus of epidemic hepatitis. Viruses recovered from dogs have been found to be responsible for lesions in the liver cells, identical to those produced by viruses in humans. However, identification of the viruses of human beings and dogs is still a problem of study. Animals play no role in the epidemiology of human epidemic hepatitis.

Pathogenesis and diseases in man. During natural infection the virus enters the alimentary tract and invades the blood and lymph. Being capable of extremely marked tropism, the causative agent produces acute diffuse hepatitis accompanied by lesions in the parenchymal and reticuloendothelial hepatic elements and by deterioration of the disintoxication function of the liver. The spleen, gall-bladder wall, and the nervous, endocrine, and other systems may be involved.

The disease is characterized by jaundice, tenderness of the liver, degenerative and necrotic processes, and subfebrile temperature. It lasts for 25-30 days. Subclinical forms of the disease as well as cases of virus carriers among healthy people occur.

The causative agent is spread by water and foodstuffs contaminated by patients and by flies. Patients and carriers discharge the virus with the faeces and urine. More than 50 per cent of all the cases occur among children.

There are two forms of epidemic hepatitis. The first form (type A epidemic infectious hepatitis) is spread by the oral-faecal route, the second form (type B serum hepatitis) develops as a result of parenteral infection. The first form has an incubation period of 15-45 days (26 days on the average), while the duration of that of the second form is longer, from 2 to 6 months.

The source of A epidemic hepatitis is a patient or carrier. The route of infection is similar to that in intestinal diseases. B serum hepa-

titis is the result of infection acquired during various injections and biological tests (injection of vaccines, sera, drugs, allergens) performed with inadequately sterilized syringes and needles previously used for injections into patients and carriers.

Immunity. The disease confers immunity of a high grade and long duration. Antibodies and gamma-globulins, capable of inactivating the virus, are present in convalescent blood. Gamma-globulins of human blood are used for prophylaxis.

Laboratory diagnosis is made by special examinations. They include liver function tests (for the presence of bilirubin and cholesterol in the blood), precipitation reactions (thymol turbidity test, sublimate test), and determination of the activity of aldolase, transaminase, etc.

Determination of transaminase activity in the patient's serum is of diagnostic value. Tests for agglutination of bacteria saturated with the virus and the complement-fixation reaction are also employed.

Treatment. There is no specific treatment. Ascorbic acid is quite effective. In cases of severe intoxication dry plasma and hormones (cortisone, hydrocortisone, etc.). are prescribed.

Prophylaxis. Epidemic hepatitis is spread by the oral-faecal route. Consequently, infection is prevented by isolating the patients, observing all contacts, performing disinfection in the foci, and by disinfecting faeces, urine and sputum of the patients and convalescents. Serum hepatitis prophylaxis is ensured by careful sterilization of needles used for taking blood, performing vaccinations, biological tests (determination of allergic conditions), and parenteral injections of drugs. Special treatment of donor blood is also necessary. Injections of gamma-globulin are recommended as measures of prophylaxis. Gamma-globulin in association with the widely distributed virus confers passive-active immunity.

TICK-BORNE ENCEPHALITIS VIRUS

The aetiological agent was discovered in 1937 by L. Zilber, M. Chumakov, E. Levkovich, V. Soloviev et al. The problems of epidemiology and, particularly, of natural nidality were studied by E. Pavlovsky and his collaborators.

Morphology. The virus is 20-40 mμ in size and passes through bacterial filters. It occurs as inclusions within the nuclei of hippocampus cells.

Cultivation. The virus is grown in tissue cultures, in the membranes of chick embryos, and in the body of white mice. The best method of cultivation is animal inoculation.

The aetiological agent of tick-borne encephalitis renders a cytopathogenic effect on the renal tissue cells of pig embryos and on some

tissue subcultures. It causes cytoplasm degeneration, pyknosis, and destruction of the nuclei.

An increase in RNA content, particularly within the degenerative cells, has been recorded. The glycogen content also increases, glycogen occurring in the form of large amorphous clusters. In the oxidation processes taking place in the cells, succino-dehydrogenase activity increases and cytochrome-oxidase activity decreases.

Antigenic structure. There are several varieties of the tick-borne encephalitis virus. They are all identical in antigenic structure and produce cross immunity. In immunological properties the virus of tick-borne encephalitis is similar to the virus of Scotch encephalitis (also known as louping ill of sheep and Scotch encephalomyelitis), virus of two-wave encephalitis (milk fever), and viruses recovered from mice.

As a result of serial passage in laboratories, the tick-borne encephalitis virus loses its pathogenicity to a considerable extent. This property is made use of in preparing vaccines (live vaccines).

Resistance. The virus survives for 70 days in 50 per cent glycerin. A temperature of 60-70°C kills it in 10-15 minutes, a 1 per cent lysol solution, in 3 minutes, a 3 per cent lysol solution, in 2 minutes, and ether and acetone, in 3 days.

Pathogenicity for animals. Chipmunks, rodents of the squirrel family, hares, thrushes, hazel-hens, and numerous other wild animals and birds which do not contract the disease but are virus carriers of long duration constitute the natural reservoir of the virus. The vectors are the *Ixodes* ticks (*Ixodes persulcatus*) which have been shown to be capable of transovarial transmission.

Laboratory mice and monkeys are susceptible to experimental intracerebral, subcutaneous, and intranasal inoculation.

Pathogenesis and diasease in man. Tick-borne encephalitis is a zoonotic disease which is transmitted from animals to man by a tick bite and by milk from virus-contaminated cows and goats. The aetiological agent invades the blood. It displays a manifest tropism to the central nervous system (the brain stem nuclei and the anterior horns of the cervical part of the spinal cord). As a result, meningoencephalopoliomyelitis develops. Virusaemia is noted during the acute stage of the disease.

Tick-borne encephalitis is accompanied by fever, somnolence followed by insomnia, sensitivity and motor disturbances, and meningeal manifestations. Complications occur such as atrophic paralysis of the shoulder and neck muscles.

Immunity. The disease leaves a high-degree and lifelong immunity. Immunity may also be produced as a result of an asymptomatic infection; this occurs in endemic areas.

Laboratory diagnosis is made by isolating the virus from the blood, performing the neutralization test on mice, and by employing the complement-fixation reaction.

Treatment is accomplished with anti-encephalitic serum which is injected intramuscularly, 40-50 ml for 2-3 consecutive days or every other day. Specific gamma-globulin is also used.

Prophylaxis. The disease is characterized by natural nidality. Zones of natural foci cover large territories in the RSFSR, Kazakhstan, Belorussia, Ukraine, and other republics of the USSR. From 4 to 12 per cent of all the ticks inhabiting natural disease foci are infected with the encephalitis virus.

Together with the typical and mild forms of encephalitis, asymptomatic infection occurs quite frequently among human beings, which is responsible for producing immunity.

Neither human beings, ticks, nor animals can contract infection from a tick-borne encephalitis patient.

Scotch encephalomyelitis of sheep (human beings are mildly susceptible to the disease and contract a mild or asymptomatic form), Australian encephalitis, and encephalomyelitis of mice are varieties of tick-borne encephalitis.

Tick-borne encephalitis control includes early diagnosis, hospitalization of patients, and protection of people from the bites of ticks. Tick extermination is necessary, particularly of those parasitic on domestic animals. Milk must be boiled. Treatment with 10 per cent DDT powder, hexachlorocyclohexan, and chlorophos should be carried out for 2 or 3 years in succession.

Specific prophylaxis is accomplished with a vaccine prepared from the tick-borne encephalitis virus passaged on chick embryos or tissue cultures. It is inactivated by formalin and freeze-dried under vacuum.

Good effect is rendered by individual prophylaxis, i.e., injection of anti-encephalitic gamma-globulin.

JAPANESE ENCEPHALITIS VIRUS

The viral nature of Japanese encephalitis was demonstrated in 1934 by M. Hayashi. Problems of aetiology and routes of transmission were studied extensively in 1938-39 by A. Smorodintseff, E. Levkovich, A. Shubladze et al.

The virus is 20-30 mμ in size.

In 1938 it was proven that Japanese encephalitis is transmitted by the bites of *Culex* and *Aëdes* mosquitoes. Rats, various species of warm-blooded animals, and birds of the sparrow family act as reservoirs of the virus. The disease occurs in Japan, Korea, China, Taiwan, the Philippines, and the Maritime Territory of the Far East.

Japanese encephalitis is characterized by deep lesions in the central nervous system, particularly in the brain stem and basal nuclei of the brain. The death rate is very high. It reached 60 per cent in Japan and 25-53 per cent during outbreaks of epidemics in the Far East (Maritime Territory).

Recovery is followed by a high-grade immunity. No complications in the form of paralysis which occur in tick-borne encephalitis are encountered.

It has been ascertained that Japanese encephalitis occurs not only in a severe form but may take a mild or asymptomatic course. In the latter case infection is accompanied by accumulation of antibodies in the blood of people living in endemic areas.

In the USSR Japanese encephalitis occurred in woodless, thinly populated territory that has a large number of lakes and swamps. There is a continuous circulation of the virus among mosquitoes and animals in endemic foci. Mosquitoes are infected with the virus for life. A transovarial mode of virus transmission has been proved. Large quantities of the virus accumulate in the mosquito body at a temperature of 27-30°C. With each bite the mosquito discharges about 100,000 lethal mouse doses. At temperatures lower than 20°C the development of the virus in the mosquito body is retarded.

Japanese encephalitis it characterized by natural nidality and seasonal prevalence (August and September).

Treatment is accomplished with anti-encephalitic serum.

Prophylaxis is ensured by enforcement of measures which protect people from mosquito bites and by vaccination.

At present vaccination against Japanese encephalitis is not practised in the USSR as there is no disease incidence.

ST. LOUIS ENCEPHALITIS VIRUS

The viral nature of encephalitis was proved in 1933 by R. Muckenfuss, C. Armstrong, and H. McCordock.

The virus is 20-30 mμ in size. It is well preserved in a frozen state and survives for more than 2 months in 50 per cent glycerin. It is somewhat related although not identical to the virus of Japanese encephalitis. The virus of St. Louis encephalitis affects the basal cerebral nuclei. In its clinical manifestations the disease is similar to Japanese encephalitis and is characterized by seasonal prevalence (July-September). It is transmitted by a bite of the *Culex* mosquito. In the autumn of 1933, 2,000 cases of encephalitis with a death rate of 20-30 per cent were registered in the USA in St. Louis County and Kansas State. Preventive measures are the same as in Japanese encephalitis.

LYMPHOCYTIC CHORIOMENINGITIS VIRUS

The virus was isolated in 1934 by C. Armstrong and R. Lillie. Choriomeningitis (acute serous meningitis) viruses are from 40 to 60 mμ in size. No inclusions have been demonstrated in this infection.

The causative agent is inactivated within 20 minutes at 56°C and within 24-48 hours at 37°C. It is destroyed by contact with soaps and is sensitive to large doses of penicillin. It remains viable for several months in glycerin and when freeze dried.

In human beings the virus affects the meninges and brain (choriomeningitis, meningoencephalitis).

The reservoir of the virus is grey mice, field voles, forest mice, and other small mouse-like rodents. Ticks (*Allodermanyssus sanguineus*, *Lyponyssus bacoti*, and *Dermacentor andersoni*) are vectors. The virus is distributed throughout the world. Human beings are infected from mice. Diagnosis is made by isolating the virus from the cerebrospinal fluid and blood and by carrying out the neutralization test and complement-fixation reaction.

VIRUSES OF HAEMORRHAGIC FEVERS

Causative Agent of Far East Haemorrhagic Fever

The virus was discovered in 1939-41 in the Far East by A. Smorodintseff and his collaborators. It is pathogenic for cats and some mouse-like rodent species. It is believed that field voles, minute mice, field mice, and rats constitute the virus reservoir. Ticks and fleas which are parasitic on these animals are presumed to be the vectors. The disease occurs in the Eastern regions of the USSR. Disease in man is accompanied by fever and haemorrhagic nephrosonephritis. There is no specific therapy or prophylaxis.

Other haemorrhagic fevers have been described (Tula, Yaroslavl, Bucovina, Uzbekistan, Manchurian, Argentinian, young swineherd's disease in Switzerland, Chiazanur forest disease in India, etc.). All these diseases differ in their clinical course and epidemiological characteristics.

Causative Agent of Crimean Haemorrhagic Fever

The virus was discovered in 1944 by M. Chumakov. It survives in 40 per cent glycerin and in the cold. The virus is virulent for cats and monkeys (Macaca rhesus), the resultant infection being accompanied by numerous haemorrhages. As was proved later, these haemorrhages can be reproduced experimentally in white mice and young rabbits.

Disease in man is characterized by fever, blood circulation disturbances and is accompanied with numerous haemorrhages in organs and body cavities (stomach, intestines), focal haemorrhages in the lungs, and haemorrhagic rash on the skin.

The disease is recognized by its clinical manifestations, epidemiological data, isolation of the virus by inoculating chick embryos

and kittens, and by employing the neutralization test and complement-fixation reaction.

Crimean fever is characterized by natural nidality. The vectors are *Ixodes* ticks of the genus *Hyalomma* (*H. anatolium*, *H. plumbeum*). These ticks multiply in great numbers in summer. They are parasitic on various rodent species, on birds, and on domestic animals, and hares in particular are infected.

Disease prevention is ensured by systematic tick disinfestation in places of their possible contact with people, hares, susliks, and other field rodents. Weeds must be burned. Ditches must be dug around field-camps and places of rest. Insect repellants (dimethylphthalate, dibutylphthalate, creosol, kerosene, petroleum, and turpentine) are recommended. The body must be protected from tick bites. Vaccination is not employed.

Causative Agent of Omsk Haemorrhagic Fever

The virus was discovered in 1947 by M. Chumakov. In morphology, filterability, and resistance this virus is similar to the aetiological agent of Crimean haemorrhagic fever. Immunologically it is identical with the causative agents of tick-borne encephalitis. Scotch encephalomyelitis of sheep, and a number of other viruses. It is easily adapted to various organs and tissues, particularly to the nerve tissue. It does not lose its immunogenic properties on exposure to formalin. The preparation of formaldehyde-treated vaccines is based on this principle.

The virus is virulent for monkeys, guinea pigs, cats, field voles, muskrats, and white mice which are readily infected by any mode of inoculation. The virus is sustained easily in the laboratory in the body of white mice infected intracerebrally and in chick embryo cultures.

Mouse-like rodents (the stenocephalic field mouse) and wild animals are a possible reservoir of the virus. The vector is the *Dermacentor pictus* tick. The virus is transmitted transovarially. The existence of natural nidality is ascertained in the forest-steppe areas of the Omsk region.

Human beings contract the disease through tick bites. Dust and alimentary routes of transmission are also possible.

Diagnosis is made on the basis of clinical, epidemiological, and laboratory data (virus isolation, virus neutralization test. complement-fixation reaction, demonstration of a rise in antibody titre during paired sera examination).

Treatment is accomplished with a specific therapeutic vaccine. General prophylaxis is ensured by protecting human beings from tick bites. Vaccine prepared from killed virus strains is used for specific prophylaxis.

Virus of Dengue Fever

The viral nature of dengue fever was ascertained in 1907 by P. Ashburn and C. Craig. The virus measures from 20 to 30 mμ and forms elementary bodies 17-25 μ in size within the cells. After adaptation to the body of mice by successive intracerebral passages, it grows readily in a chicken embryo. Two types of viruses have been disclosed. The virus contains the thermostable and thermolabile antigens. The latter causes a group complement-fixation reaction with the viruses of yellow fever and Japanese and West Nile encephalitides.

The virus persists for a period of 5 years at a temperature of —70°C and in a dried state and remains viable for 2 months in patient's serum at room temperature. It dies very quickly on exposure to light and is nonresistant to heating. Weak bile dilutions (1:10, 1:15) inactivate it in 5 minutes and ultraviolet rays and an 0.05 per cent formalin solution destroy it.

The virus is poorly pathogenic for laboratory animals. Adapted strains cause paralysis and death in albino mice and virusaemia in guinea pigs. Infection of Macaca rhesus monkeys results in a mild form of the disease.

The virus possesses toxic activity. It affects the neurons in the cerebrum and spinal cord and causes degenerative changes in the cells of the liver, kidneys, and heart. It produces haemorrhagic lesions in the endocardium, pericardium, gastric and intestinal mucosa, peritoneum, central nervous system, muscles, and skin. Deep disorders are revealed in the small blood vessels (swelling of the endothelium, perivascular oedema, and infiltration by mononuclear cells).

The sources of infection are sick people. The virus appears in the patient's blood during the latter 24 hours of the incubation period and remains there for 3 or 4 days of the febrile period. Infection occurs through the bite of *Aëdes aegypti*, *Aëdes albopictus*, and *Aëdes scutellaris* mosquitoes. At a temperature of 22°C the mosquito becomes capable of transmitting the virus in 8-12 days after a meal on the patient's blood. At 16°C the causative agent does not develop within the mosquito's body. The mosquito remains infective for a period of 174 days.

The incubation period in dengue fever varies in duration from 2.5 to 15 days, lasting 5-8 days on the average. Quite frequently the disease has a sudden onset with chills, headache, severe pains in the joints, muscles, and eyeballs, and a high fever (39-41°C). The face becomes crimson and the sclerae injected. Erythema may be encountered in some patients. A remission occurs in 1-4 days. The temperature drops and the body becomes covered with profuse perspiration. This is followed by a second attack which is characterized by an elevation of temperature and the presence of the same

symptoms as in the first attack. A maculopapular or scarlatina-like eruption appears on the body, lasting not longer than 3 or 4 days. The duration of the disease is 4 or 5 days.

During epidemics mild and severe forms of the disease are encountered along with the typical form. They are marked by coma. delirium, convulsions, and mucopurulent diarrhoea. The lethality rate is low, the patient usually recovers.

Complications include myelitis, polyneuritis, otitis, parotitis, lymphadenitis, postinfectious psychosis, and keratitis.

The disease leaves an immunity which lasts 2 to 6 months.

Diagnosis rests on clinical, epidemiological, and laboratory findings. The virus is isolated from the blood in the first days of the disease by intracerebral inoculation of mouse sucklings (not over 3 days of age), and the complement-fixation reaction and the neutralization test are performed.

There is no specific therapy. Symptomatic remedies are used: large amount of liquid is given to drink, a 10 per cent glucose solution is injected intravenously, and amidopyrine, acetylsalicylic acid, preparations of iron, and vitamins C, B_1, and B_2 are given.

Dengue fever occurs as an endemic disease in regions with a tropical and subtropical climate. It is not encountered beyond the latitudes 42° North and 40° South, and does not occur in the USSR. It was responsible for large epidemics. More than 500,000 people suffered from the disease in Texas in 1922. In 1925-26 560,000 cases were registered in Queensland and New South Wales. Eighty to ninety per cent of the total population of Greece contracted the disease in 1927-28. A large outbreak occurred in Japan among the civil population and the USA army during World War II.

Prophylaxis comprises isolation of patients, prevention of access of the vectors to them, extermination of mosquitoes, and protection from their bites. Quarantine measures are enforced to prevent the spread of the infection to countries free from the disease. Measures of specific prophylaxis are still being elaborated.

Virus of Yellow Fever

The viral theory of the origin of yellow fever was suggested by the Cuban scientist K. Finley in 1881. The virus was discovered in 1901 by the American investigators W. Reed, J. Carroll, J. Agramonte, and J. Lazear.

Morphology. The virus ranges from 12 to 27 mμ in size and forms irregular-shaped inclusions within the nuclei of nerve and hepatic cells.

Cultivation. The virus grows in the brain tissue of albino mice and Macaca rhesus monkeys. It also multiplies readily in ground mouse embryo tissue cultures, in a developing chicken embryo, in mouse testicular tissue, and in a monkey kidney tissue culture.

Resistance. The virus is highly resistant to high temperatures. It persists for 3 months in 50 per cent glycerin on ice, for more than a year in a frozen state, and for 12 years in a dried state. It is sensitive to heating and is destroyed at 55-65°C in 10 minutes. It is very rapidly killed by exposure to 0.5 per cent formalin and other disinfectants.

Pathogenicity for animals. In natural foci the virus of yellow fever lives in the body of animals of more than 27 species (monkeys, opossums, armadilloes, etc.). Among the experimental animals those susceptible to the disease are the European hedgehogs, albino mice, Macaca rhesus monkeys, and others. The virus is transmitted from one animal to another by the *Haemogogus* mosquito which breeds in the hollow of a tree or on the soil surface in the tropical forests. In 1-18 days the infected mosquitoes become capable of transmitting the infection and remain infective throughout life (70-116 days).

Pathogenesis and disease in man. Yellow fever occurs in two forms. The urban yellow fever is transmitted from a sick person to a healthy one by *Aëdes aegypti*, *Aëdes simpsoni*, *Aëdes calopus*, *Aëdes africans*, and other species of mosquitoes. The *Aëdes aegypti* mosquito, however, is known as the main vector. The jungle yellow fever is transmitted to man from wild animals and rodents through mosquito bites. The disease prevails among people working in the forest. The virus penetrates the reticulum cells of the lymph nodes and then invades the blood, liver, spleen and bone marrow. It is found in the human blood up to the fifth day of the disease but later is located in the lymph nodes. The virus causes necrotic and fatty degeneration of cells in the liver, kidneys, and spleen, and gives rise to haemorrhages in the internal organs (stomach, pleura, lungs, and intestinal mucosa). The main clinical symptoms of yellow fever are jaundice, haemorrhages, and intensive albuminuria.

The incubation period lasts 3 to 6 days. The disease is characterized by a sudden onset and runs in cycles. The first (infectious) phase is manifested by headache, pain at the back of the head and in the muscles of the back, and flushed skin, conjunctiva, and mucous membranes. Nausea, anorexia, thirst, and polyuria are encountered at the onset of the disease. In the second phase (the period of remission) by the end of the third day there is a fall in temperature, sometimes below normal, and relative well-being sets in. In mild cases the disease terminates in recovery in this phase. The third phase is characterized by intoxication, disappearance of the virus from the peripheral blood, massive gastrointestinal haemorrhages, anuria, and by an increase in the amount of protein and hyaline and granular casts in the urine. A hepatic or uraemic coma develops in fulminant forms of the disease and results in death.

With regard to the degree of severity, asymptomatic, very mild, benign, malignant, and fulminant forms of the disease are distinguished.

Complications include abscesses, parotitis, pneumonia, multiple skin lesions, sometimes gangrene, and myocarditis.

Immunity is associated with the presence of virus-neutralyzing antibodies in the blood, which form on the fifth day of the disease. They are produced over a period of several weeks. With time, the antibody titre diminishes gradually and persists at a low level for many years following recovery.

Laboratory diagnosis. The diagnosis of yellow fever is based on clinical, epidemiologic, and laboratory data. Laboratory examination comprises isolation of the virus from the patient, detection of specific antibodies in the blood of patients and convalescents, and a histologic study of the liver in lethal cases. The virus is isolated by intracerebral inoculation of albino mice with patient's blood collected in the first 5 days of fever. If the virus is present in the blood, symptoms of encephalitis will develop in the animals in 7-20 days following the inoculation. The presence of virus-neutralizing antibodies is detected by performing the neutralization test, and the haemagglutination-inhibition and complement-fixation reactions.

Treatment. No specific therapy is known. Bed-rest, thorough nursing care, and symptomatic treatment (administration of glucose, calcium gluconate, alkaline solutions, vitamins, purgatives, and cardiacs, transfusion of blood and plasma) are recommended.

Prophylaxis. Yellow fever is a menacing disease which had caused the death of enormous numbers of people in the past. It has been known since 1648 among people living on the islands in the Caribbean Sea and along the coast of North, Central, and South America. From 1798 to 1900 500,000 people in America contracted the disease. Yellow fever caused 36,000 deaths in Havana in 1853-1900, 23,000 deaths in Rio de Janeiro in 1851-83, and 22,000 deaths in 1881-89 during the building of the Panama Canal.

Yellow fever control is effected by general measures elaborated by C. Finley in 1894 (extermination of mosquitoes, protection from their bites, disinfestation of all transport arriving from endemic regions). However, active immunization with a live vaccine obtained by M. Theiler in 1936 proved to be the most effective measure of prophylaxis. Two vaccine strains are used for vaccination: the American and the French. The American vaccine is obtained from the tissues of infected chicken embryos, dried under vacuum, and stored in a frozen state in ampoules which are filled after being dried with nitrogen. Before use the vaccine is dissolved in a physiologic solution. It is injected subcutaneously in a dose of 0.5 ml. The French vaccine was prepared in 1939 by M. Peltier and his colleagues from a virus isolated from a patient in Dakar (West Africa) in 1929 and attenuated by prolonged intracerebral passages in albino mice. It is produced as a dry vaccine from dried brain tissue of infected mice. The vaccine is introduced through three scarifications made on the skin and may be applied together with the

smallpox vaccine. The American vaccine causes a less manifest reaction but its vaccination rate is sometimes low (67-70 per cent). The French vaccine, on the other hand, gives a more pronounced reaction and is highly immunogenic (97 per cent). Vaccination is performed once in 4 years. The incidence of yellow fever has almost been liquidated in Equatorial Africa due to a wide use of the French vaccine.

However, foci of yellow fever still exist in the central regions of South America in spite of the fact that a highly efficient vaccine is available. This disease causes epidemics in South Africa (Senegal, the Gold Coast, certain regions in the Congo). It occurs in Sudan (East Africa). In the countries of America and Africa 2,927 cases were registered during the period from 1950 to 1958, and 3,507 cases in 1959-64.

Sandfly Fever Virus

The causative agent was discovered in 1909 by R. Doerr, K. Franz, and G. Taussig.

The virus is 20-40 mμ in size. It is killed by heat at 56°C in 10 minutes and survives in glycerin for up to 2 weeks. Laboratory animals are unsusceptible to the virus. The causative agent may be grown on the chorioallantoic membrane of a chick embryo and in tissue cultures.

A disease, known as pappataci fever, was found to occur on the Mediterranean coast, in some provinces of India, in Africa, Central and South America, in Crimea, Caucasus, Odessa region (previously known as Ismail region), and in Central Asia. The condition is characterized by fever, pains in the forehead and orbits, muscular pains, a burning sensation in the eyes, and redness of the conjunctiva. The disease lasts for 3 days and leaves a weakness and lassitude of several days' duration. All patients recover from sandfly fever.

The disease is spread by *Phlebotomus pappatasii*. The virus has a definite cycle of development in the sandfly body and is transmitted by the transovarial route. The sandfly becomes infective only on the seventh day after biting a patient suffering from the fever.

Sandfly larvae survive in dark damp places rich in organic matter, and in animal burrows. Sandflies attack human beings during the night; in the daytime they seek refuge in unused premises, ruins, and broken bricks.

Immunity is delayed. No high-grade or lasting immunity is produced by a single attack of the disease. Only re-infection leaves a high-grade immunity.

Sandfly control is ensured by the use of DDT, hexachlorocyclohexan, and chlorophos. General sanitary measures must also be

carried out (clearing yards and living premises of rubbish). As a result of enforcement of regular and active measures, sandfly fever incidence has been reduced to single cases in the USSR.

RABIES VIRUS

V. Babes in 1892 and A. Negri in 1903 described specific inclusion bodies in nerve tissue cells. These bodies are referred to as Babes-Negri bodies. The causative agent of rabies is a neurotropic virus. It has a selective affinity for the nervous system and is discharged with the saliva.

Morphology. The virus ranges from 100 to 150 mμ in size and is spherical in shape. The Babes-Negri bodies vary from 1 to 10 μ. They may be spherical, oval, or polygonal and occur within the nerve cell cytoplasm and dendrons. The bodies readily stain red by the Mann and Romanowsky-Giemsa methods, while the nerve cell cytoplasm stains light-blue (see Fig. 127, *8*). The Babes-Negri bodies are found in 98 per cent of street rabies cases being more frequently within the cells of the Ammon's horn and in Purkinje's cells of the cerebellum.

Cultivation. The rabies virus multiplies on embryonal brain tissue of the mouse, chick, rabbit, guinea pig, and white rat. Recently the street rabies virus has been adapted to the chick embryo by inoculating it into the yolk sac or directly into the brain of an embryo. The rabies virus is quite easily adapted to cancer tumours of various animal species and this is evidence that its neurotropism is not absolute.

Antigenic structure. Studies of the antigenic structure, carried out over many years, have shown that no special varieties or types exist within the species. No significant difference between the street virus and the fixed virus has been revealed (see p. 151). Their antigenic structure was proved to be identical, the current rabies prophylaxis being based on this fact.

The fixed virus is a variety of the rabies virus (see p. 151). As distinct from the street virus, the fixed virus forms no Babes-Negri bodies as a rule and is highly pathogenic for rabbits on intracerebral inoculation.

A subcutaneous inoculation of the fixed virus is of low pathogenicity or completely nonpathogenic for human beings. These properties are stable. Persons bitten by rabid animals have successfully been vaccinated with the fixed virus at all Pasteur stations for 80 years.

Classification. All the strains of the street virus which have been isolated and studied are subdivided into three groups: (1) strains of the classic rabies virus which include the majority of viruses isolated from human beings and animals; (2) "intensified" strains

of the street virus, characterized by high pathogenicity, against which vaccination does not always afford protection; (3) strains of the street virus which produce atypical forms of the disease. Besides this, the following types of street virus strains are distinguished: (a) those that relatively quickly transform to the fixed variant, (b) those that are fixed after numerous passages on animals, and (c) those that are stable in their hereditary properties. New biological variants can be formed under natural conditions when the virus passes through the body of a host not natural to it, i.e., that of blood-sucking vampires.

Resistance. The rabies virus is resistant to low temperatures and glycerin. It persists for a long time in nerve tissue and sometimes even after the animal dies. The virus is inactivated at 60°C in 10-15 minutes and on exposure to direct sunlight and ultraviolet rays. It is destroyed by a 5 per cent phenol solution and by other disinfectants.

Pathogenicity for animals. Rabies is prevalent among dogs, wolves, and foxes and occurs less frequently among cattle, horses, sheep, swine, deer, cats, rats, mice, and birds. The virus is transmitted by the bites of rabid animals and when the saliva becomes transferred onto scratches and abrasions on the skin and mucous membranes.

Pathogenesis and disease in man. There is an interval of from 15 to 45 days, sometimes 3 months, and in some cases over a year from the moment of infection to the onset of the disease. The duration of the incubation period depends upon the amount and virulence of the virus introduced and upon the nature of the affected tissue. The disease is characterized by a severe clinical picture and 100 per cent mortality (world literature has described only one case of recovery from rabies).

From the site of penetration (a wound or abrasion) the rabies virus moves along the nerves with the fluid in the perineural spaces and reaches the central nervous system. It may enter the central nervous system with the blood and lymph.

The largest amount of virus accumulates in the Ammon's horn, medulla oblongata, cerebellum, cranial nerve nuclei, sympathetic ganglions, and in the lumbar part of the spinal cord. The involvement of these vitally important parts of the nervous system results in excess reflex excitability and convulsions, particularly in the deglutitory and respiratory muscles, and increased salivation and perspiration. The patient dies within 5 or 7 days displaying symptoms of aerophobia, hydrophobia, paralysis, and convulsions.

Immunity. As is known, rabies affords no postinfection immunity. The mechanism of immunity acquired after vaccination against rabies is still a problem under investigation. The majority of investigators maintain that the presence of antibodies is no direct criterion of vaccination immunity, although the use of seroprophy-

laxis and gamma-globulin in combination with vaccination against rabies is very effective, particularly in severe bites. Interference between the street virus and the fixed virus probably plays the main role in postvaccinal immunity.

Laboratory diagnosis includes: (1) demonstration of Babes-Negri bodies in cells of Ammon's horn on post-mortem examination of human beings and animals; (2) intramuscular or subdural inoculation of white mice, cotton rats, rabbits, or guinea pigs with an emulsion of the brain of dead human beings and animals. Paralysis appears in 2 or 3 weeks; (3) autopsy of the stomach of dogs that had died of rabies, for revealing the presence of foreign bodies (rags, stones, chips of wood, etc.); (4) intracerebral inoculation of white mice with animal or human saliva treated with antibiotics. This method is used for in vivo diagnosis.

Treatment. There is no known treatment for rabies at present. No specific method has been worked out.

Prophylaxis is ensured by a complex of general and specific measures. They include: (a) destruction of rabid animals and stray dogs, registration of all owned dogs, enforcement of dog muzzles, barring the transfer of dogs from places endemic in regard to rabies, and systematic health education; (b) immediate injection of antirabic gamma-globulin into the person bitten by a rabid or suspected animal, followed by a course of vaccination with Fermi or Phillipps' vaccine; (c) hospitalization of people severely bitten by rabid or suspected animals and the complete course of preventive inoculation given in hospital conditions; (d) enforcement of compulsory immunization of all licensed dogs.

The antirabic vaccine is prepared from the fixed virus which, as a rule, is harmless to animals on subcutaneous injection. Since the incubation period in human beings is of long duration (from 15 to 45 days and longer), intensive introduction of the vaccine inhibits the street virus.

It is thought that there is interference between the vaccine strain and the street virus, the former blocking the cells which are more susceptible to the street rabies virus. As a result of this antagonistic interaction the street virus perishes.

Apparently the virus is destroyed as it moves towards the central nervous system.

The vaccine is obtained by intracerebral inoculation of rabbits or sheep with the fixed virus. The animals are killed several days after inoculation and a 5 per cent emulsion of their brain is prepared in a sodium chloride solution. The emulsion is then treated either with phenol as described by Fermi (the vaccine contains 1 per cent phenol) or with glycerin according to the method of Phillipps (a 10 per cent emulsion).

Injections are administered subcutaneously according to a standard procedure to all humans (and sometimes to animals) who have

been bitten by rabid or suspected animals or have been contaminated with their saliva.

Postvaccinal immunity develops in 2 weeks and lasts for 6 months.

A good result is obtained with antirabic gamma-globulin which is prepared by horse hyperimmunization with the fixed virus. The immune gamma-globulin is capable of inactivating the street virus. It prevents development of postvaccinal encephalites. Gamma-globulin must be injected within 72 hours following the bite. Vaccination is commenced 24 hours after this injection.

The wound is thoroughly cleaned, and surgical debridement, washing with a 20 per cent soap solution or painting with tincture of iodine are employed. All these measures by no means exclude vaccination.

Vaccine treatment must not be carried out if there is no sufficient evidence for its necessity. Intensive injections of the fixed virus may occasionally give rise to extremely severe complications in the form of postvaccinal paralysis and may even be fatal.

Complications following antirabic vaccination include paralytic rabies, encephalomyelitis caused by the fixed virus, and allergic encephalomyelitis. The first two conditions are extremely rare but fatal. Allergic encephalitis displays a variety of symptoms of meningitis, encephalitis, myelitis, polyradiculitis, and polyneuritis accompanied by pareses and paralyses.

For the prevention of complications, since 1964 the Fermi and Phillipps vaccines have been replaced by an anallergic antirabic vaccine. It is prepared from the brain of white rat sucklings inoculated with the fixed virus. The preparation is produced as a dry vaccine. It produces no side effects and is more immunogenic than the previous preparations. The anallergic antirabic vaccine is a 5 per cent suspension of the brain of white rat sucklings, inactivated with phenol.

PAPILLOMA AND POLYOMA VIRUSES

Papilloma, polyoma, and vacuolization viruses give rise to a number of diseases including papillomas of rabbits, dogs, cows, and monkeys, human skin papilloma, human condyloma, and human larynx papilloma.

Electron microscopy shows that they are spherical in shape and vary in size: from 30-40 mμ (rabbit papilloma and polyoma viruses) to 220-260 mμ (rabbit fibroma virus). The viruses are composed of nucleotide surrounded by a multilayered membrane. They are identical to the known viruses in cultural and other properties.

Infectious warts (W. Holmes, 1948) are caused by a virus which forms spherical bodies consisting of crystalline agglomerations. Eosinophilic intranuclear inclusions may be demonstrated in this disease. Infection takes place as a result of direct contact with the

patient or through objects of common use, i.e., sports equipment, instruments, etc. The incubation period is of long duration, from 4 to 5 months. The clinical manifestations of warts vary (flat common warts, pointed condylomas, papillomas of the mouth mucosa). The disease is generally widespread and prevails in children and juveniles. Treatment is accomplished by cauterization with lapis and oral prescription of methionine.

The virus of molluscum contagiosum (W. Holmes, 1948) forms acidophilic intracellular inclusion bodies. In human beings it produces small hard semispherical nodules which on being pressed discharge a white caseous mass containing ovoid bodies ("molluscum bodies"). The nodules appear on the genitalia, pubis, abdomen, and umbilicus in adults and on the eyelids, face and neck in children. In some cases the lesions are localized on the lips, tongue, and cheeks.

Infection is conveyed by the patient through general contact or via the genital organs. Animals are insusceptible to the disease. Treatment is accomplished by evacuating the nodule contents with pincers and then painting the cyst with a 10 per cent iodine tincture or a 10 per cent silver nitrate solution.

Prophylaxis is accomplished by general sanitary-hygienic measures. Patients are not allowed to have sexual intercourse or everyday contact with healthy individuals until complete recovery. They are also not allowed to bathe in swimming pools and use common baths. Bed-clothes and beds must be for individual use.

Human skin papilloma is a circumscribed tumour of the tegumentary epithelium having the appearance of papilliform growths protruding from the skin surface. Papilloma occurs in all organs that contain tegumentary epithelium (skin, mucous membranes in the mouth, larynx, and respiratory and urogenital tracts and, sometimes, in the serous membranes). Papillomas may be single or numerous. Diffuse papillomatosis in the mucous membranes of the larynx or urinary bladder or on the skin occurs relatively rarely. With this condition the surface of the area involved is completely covered with papilliform outgrowths.

The clinical manifestations of papillomas display extreme variability. Some remain benign for many years and sometimes disappear spontaneously. Others may become malignant and be responsible for stenosis in the respiratory tract, aphonia involving the vocal cords, haemorrhages which cause anaemia, ulcerations on papillomas in the urinary tract, and proliferation into bordering organs in papilloma of the ovaries. Papillomas of the skin and mucous membranes may, in a number of cases, undergo malignant changes and develop into malignant tumours and for this reason they are regarded as precancerous conditions.

Animal and human papilloma viruses differ in antigenic structure. No cross immunity has been ascertained between the various

papilloma viruses. The possibility of infection of healthy people with cell-free papilloma filtrates has been proved in experiments on volunteers and this is indicative of the viral aetiology of these diseases.

Papillomas are treated surgically and by applying X-ray therapy and chemotherapy. Intravenous injections of novocain and painting of the larynx with antiverrucin, podophyllin, Gordeyev's solution and celandine extract are used as auxiliary measures in cases with papilloma of the larynx. Tracheotomy is necessary in cases of asphyxia.

OTHER VIRUSES

The number of viruses discovered increases with every year. Thus, viruses of the salivary glands of mice, rats, guinea pigs, minks, hamsters, monkeys, silver-black foxes, and human beings have already been studied. The human salivary gland viruses are responsible for anaemia, erythroblastaemia, thrombocytopenia, hepatosplenomegalia, development of petechiae and ecchymoses on the skin and mucous membranes, and for other diseases. In a number of cases they may be similar to haemolytic anaemia of the newborn caused by Rh-incompatibility. Virus diseases of the salivary glands in newborn infants terminate in death. Histopathologic examination reveals the presence of intranuclear inclusion bodies in the salivary gland cells.

Viruses of congenital defects of development have been recognized. They are responsible for a high mortality percentage. Congenital defects may be caused by the viruses of rubeola, measles, chickenpox, epidemic parotitis, etc.

The isolation of a virus from monkey kidneys (SV40) is of particular interest. In experiments this virus has been shown to be tumour-producing. Its presence was revealed in tissue cultures used for growing the poliomyelitis virus and in live and killed poliomyelitis vaccines.

Owing to the rapid development of virology, practical medicine has obtained new and very effective vaccine preparations against poliomyelitis, epidemic parotitis, measles, and other diseases.

At present the attention of virologists is attracted by such important problems as the structure of viruses, their interrelation with the tissue cells and protoplasts, the synthesis of virus components, the mechanism of virus reproduction, virus genetics, pathogenesis and clinics of viral diseases, the search for new drugs, and the study of the mechanism of immunity and its reproduction for carrying out specific prophylaxis. Great attention is given to the study of latent and tumour-producing viruses and of mixed viral-bacterial infections.

Virus-like particles have been demonstrated in the blood and bone marrow of leukosis patients, sometimes with a very high con-

centration in the plasma. Antibodies which neutralize the virus of mouse leukosis have been found to be present in a number of human beings affected with leukosis. Filterable agents recovered from humans are identical in character and antigenic properties to the viruses of chicken and mouse leukoses. In the process of cell transformation induced by tumour-producing viruses the genetic properties of the cells significantly alter.

According to the virogenetic theory, viruses are capable of transforming a normal cell to a tumour cell. It is believed that the latent viruses on invading the cells may cause this transformation.

Thus, it may be assumed that there is a wide variety of aetiological factors of leukoses and cancer: viruses, carcinogenic substances, the effect of temperature and radiation, etc. The elective multiplication of mutant cells and their displacement of the normal cells play an essential part in the development of malignant tumours.

Disorder of the chromosome apparatus is noted in leukosis and cancer. For example, disorders of chromosomes 6-12, 13-15, and 21-22 have been detected in leukosis, and defects of chromosome 13 in cancer. Chronic myeloid leukosis is characterized by defects in chromosomes 21 or 22.

PATHOGENIC FUNGI

Information on the morphology, structure, and classification of fungi is given in the section of this book dealing with general problems.

The fermentative properties are varied and inconstant. No soluble toxins are produced.

ASPERGILLI

The pathogenic and conditionally pathogenic species of *Aspergillus* (Micheli, 1729) include *A. fumigatus*, *A. candidus*, *A. roseus*, *A. flavus*, *A. nigricans*, etc. More than 40 species have been described. They have been recovered from patients suffering from various clinical forms of aspergillosis. In human beings they are responsible for lesions in the lungs, bronchi, cornea, ear canal, and other organs and tissues. The disease prevails among millers, ragmen, etc.

PENICILLI

There are more than 30 species of *Penicillium* (Link, 1809) pathogenic for human beings. They cause penicilliosis, involving the skin, nails, ears, upper respiratory tract and lungs (pseudotuberculosis). A general infection resulting in the development of foci in the internal organs may also develop. The pathogenic species are: *P. minutum*, *P. citrorosum*, *P. linguae*, *P. glaucum*, and *P. album*.

MUCOR FUNGI

More than 15 species of *Mucor fungi* are responsible for mucormycosis and include *M. mucedo*, (C. Linne, 1764), *M. racemosus*, *M. exitiosus*, etc. The resulting infection constitutes lung lesions

similar to tuberculosis in their clinical manifestations (pseudotuberculosis), keratitis, otomycosis, vulvovaginitis, gummas of the skin, and pseudotuberculosis of the liver.

Laboratory diagnosis of aspergillosis, penicilliosis, and mucormycosis is made by microscopic examination of the pus and its inoculation into common nutrient media or Sabouraud's medium and subsequent cultivation at 25-28°C.

Treatment is accomplished with nystatin, iodine preparations, and, in chronic cases, with autovaccines.

IMPERFECT FUNGI

Sporotrichum (Schenck, 1898) has a septate mycelium. Lateral branches extend from the hyphae, bearing single conidia or clusters of conidia (Fig. 135) at their sides or ends. The *Sporotrichum*

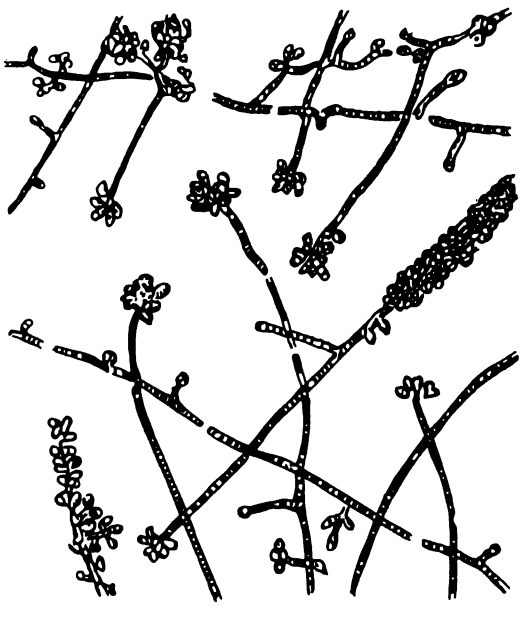


Fig. 135. Sporotrichum

grows on common nutrient media (pH 6.5) producing the best growth on Sabouraud's medium at 25-28°C. Growth is slow. The colonies are leathery, fluffy, smooth or folded and quite frequently pigmented.

The causative agent enters the subcutaneous cellular tissue and the lymph nodes through abrasions in the skin. As a result, small gummas resembling those in syphilis and tuberculosis are produced in the pharynx, larynx, muscles, and synovial membranes. The fungus may also be the cause of abscesses in the bones, joints, muscles, and internal organs.

Laboratory diagnosis is made by microscopic examination of pathological material (pus, sections of biopsy specimens) obtained from the patient. Diagnosis is confirmed by the presence of Gram-positive fungi observable as ovoid bodies 1×2-$1\times3\mu$ in size.

The pure culture is isolated by inoculating the material into Sabouraud's medium.

Agglutinins in a titre of 1:200-1:500 are detected in patients' sera as well as precipitins, opsonins, and complement-fixing antibodies.

An intracutaneous injection of fungal culture extracts produces a local allergic reaction in patients. *Sporotrichum* is pathogenic for animals. A subcutaneous injection into mice, rats, and guinea pigs gives rise to granulomatous lesions in the internal organs.

Sporotrichum is widely distributed in the external environment. It occurs in dust, on plants, on the skin of human beings and animals, and on various objects.

Treatment is carried out by means of iodine preparations, vaccines, autovaccines, and X-rays. Antibiotics and sulphonamides have no effect. A positive effect has been obtained with badional (sulphathiocarbamide, phontamide).

Prophylactic measures against sporotrichosis have not been worked out. The rules of hygiene should be observed (body hygiene and prevention of skin and mucosal injuries).

Causative agents of candidiasis. These include yeast-like fungi of the genus *Candida*. They are unicellular organisms (Fig. 136) which reproduce by budding. Neither conidia nor ascospores are produced. They possess no true mycelium; the pseudomycelium is devoid of a membrane and septa and develops by successive or terminal budding.

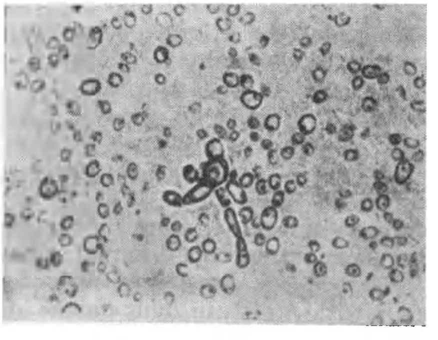


Fig. 136. *Candida*

Candidiases (M. Berkhaut, 1923) caused by *C. albicans* and *C. tropicalis* have lately been of most importance in human pathology. About 20 species of the genus *Candida* are pathogenic for human beings. These fungi are present on the skin and mucous mem-

branes of the mouth, gastrointestinal tract and urogenital organs of man. They occur on fruit, vegetables, and other foodstuffs, in bath sewage, washings from catering establishments, and on dishes and various objects.

The infection is acquired when the body is weakened and in the presence of unfavourable conditions (increased moisture, skin maceration, long-term occupational contact with sugar-containing fruit and vegetables, and many other factors). Infection through inadequately disinfected baths is also possible. The disease occurs among confectioners, bath-house attendants, and debilitated children.

Candidiases associated with long-term use of antibiotics (penicillin, penicillin-like preparations, chlortetracycline, etc.) have become of great importance. They cause profound disturbances of symbiotic relationships among normal microflora, resulting in dysbacteriosis.

The condition of dysbacteriosis enhances intensive multiplication and spread of some of the co-members of the intestinal, mucosal, and skin biocoenosis and their transformation from a saprophyte state into conditionally pathogenic and pathogenic forms. As a result, local and general lesions develop.

The yeast-like fungi affect the skin between the fingers and toes and in the inguinal and axillary folds, the nails and nail folds, the mucous membranes of the lips and mouth corners, the tongue, fauces, oesophagus, and, sometimes, the vagina with the development of white films.

The gastrointestinal and respiratory tracts and the urogenital and nervous systems are also involved in candidiasis. Candidiasis of the liver, biliary tract, pancreas, and bones also occurs. In a number of cases the causative agent may be responsible for a septic process which becomes chronic and is characterized by lesions in the kidneys, lung tissue, liver, and other organs.

The causative agents of profound forms of blastomycosis include fungi of the genera *Blastomyces*, *Cryptococcus*, etc.

Blastomyces dermatitidis (T. Gilchrist, W. Stokes, 1898) can be seen under the microscope as large budding cells. Its mycelium is segmented, branching, and with conidia protruding laterally. On blood agar at 37°C the fungus produces white, moist, waxy, soft, wrinkled colonies of the yeast type. On Sabouraud's medium at 25°C it produces fluffy white colonies (Fig. 137) which turn brown.

In human beings the fungus is responsible for deep-seated (North American) blastomycosis of the Gilchrist type. The disease becomes chronic and skin lesions on the face, hands, and buttocks develop. Involvement of the internal organs is relatively rare.

Blastomyces brasiliensis (A. Splendore, 1912) occurs as large cells with multiple buds on the surface. On blood agar it produces yeast-like and cerebriform colonies. On Sabouraud's agar it produces white velvety colonies which later become bulging, convex,

and brown in colour. In human beings the fungus produces South American blastomycosis, a severe chronic infection. The condition is characterized by granulomatous lesions in the skin, mucous membranes, lymph nodes, and internal organs.

Cryptococcus neoformans (F. Sanfelice, 1894) occurs as round and oval yeast-like cells with one or two buds, which are frequently surrounded by a capsule. On Sabouraud's medium it forms mucoid white colonies which later turn cream and brown in colour.

In patients' pus, sputum, cerebrospinal fluid, and tissues the fungus measures 5 to 20μ in diameter, has one bud each, and is surrounded by a wide capsule.

In human beings the causative agent produces a deep-seated, systemic, chronic (European) blastomycosis of the Busse-Bushke type. The lungs, intestines, skin, subcutaneous cellular tissue, lymph nodes, bones, brain, and meninges are involved. Mortality is very high. The disease occurs among agricultural workers, swineherds, and cattle breeders not only in Europe but in America as well.

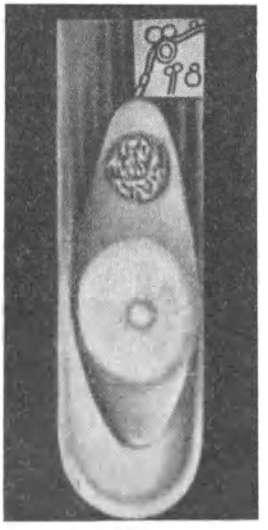


Fig. 137. *Blastomyces dermatitidis* mycelium and colonies

Laboratory diagnosis of blastomycosis includes microscopic examination of the isolated fungi and study of their cultural, biochemical, and pathogenic properties. The agglutination and complement-fixation reactions and the allergic tests must also be carried out.

Effective treatment of patients with blastomycosis and candidiasis calls for elimination of dysbacteriosis by discontinuing broad-spectrum antibiotics. Special antibiotics (nystatin, neomycin, and candicidin) are prescribed. Sulphadimezin, antiphagin (a filtrate of a two-day *Candida* fungi culture), and vaccine therapy are used. It is also necessary to recommend a full-value diet containing the required amount of vitamins and to add any required hormones. Surface candidiasis lesions are treated with 1-2 per cent iodine solutions, and 10 per cent potassium carbonate mouthwash. The affected area is painted with 10 per cent boric vaseline, a 1 per cent malachite green solution, and glycerin Lugol's solution and washed with 2 per cent sodium borate, 10 per cent sodium bicarbonate, and other antiseptic preparations.

Treatment of visceral candidiasis is accomplished with injections of iodides, antibiotic therapy (nystatin, candicidin) and immunotherapy (polyvalent, monovalent vaccines, and autovaccines prepared from *Candida* fungi killed by exposure to 70°C for 1 hour).

Prophylaxis of blastomycosis, candidiasis, and related diseases is ensured by general hygienic preventive measures (timely recognition of patients suffering from the disease, their isolation and treatment, removal of factors conducive to the spread of the infection in communities, particularly in children's institutions). Personal prophylactic measures and the correct diet regime must be observed. There must be a sufficient supply of vitamins. The teeth should be inspected and proper hygienic care of the mouth should be carried out. The skin must also be properly taken care of and excessive moisture and macerations should be treated. The use of ineffective broad-spectrum antibiotics should be banned. Measures should be taken to strengthen the physical condition of the body.

DERMATOPHYTES

The causative agents of favus, trichophytosis, microsporosis, and epidermophytosis are of the most importance among the numerous group of dermatophytes.

The causative agent of favus, *Achorion schoenleini* (I. Schoenlein, 1839), is recognized by its mycelium ends in the form of antlers of a deer, a chandelier, or a mace. It multiplies by means of chlamydospores; in flour-like cultures the mycelium displays well-defined aleuria (aleurospores produced by cytoplasmatic condensation of the mycelium).

When cultivated on Sabouraud's medium, the fungus produces dry, wrinkled, dome-like, morel-like colonies (Fig. 138) grey-yellow or brown in colour, and with a waxy or floury surface.

The causative agent of trichophytosis, *Trichophyton violaceum* (M. Gruby, 1843; R. Sabouraud, 1902), is characterized by the presence of thin, short-branched, and segmented mycelial filaments arranged at right angles to each other. In old cultures the mycelium is broader, bead-like, and contains chlamydospores. Cultures grown on Sabouraud's medium are dome-like, leathery, and smooth (Fig. 139) with a moist and, often, glistening surface. The colonies may also be smooth and wrinkled and with radial grooves. They may be violet, black, pink, or crimson in colour.

The causative agent of microsporosis, *Microsporum lanosum* (M. Gruby, 1844; R. Sabouraud, 1907), in cultures on nutrient medium appears as large pointed spindles with 5-12 cells and a jagged capsule (Fig. 140). The mycelium is raquet-like in appearance. On Sabouraud's medium the cultures are white or slightly yellowish, fluffy, and sometimes have radial or concentric grooves.

The causative agent of epidermophytosis Kaufmann-Wolf, *Epidermophyton Kaufmann-Wolf* (Kebner, 1864; Priestly, 1917), has a long and septate mycelium and spherical aleuria arranged in clusters and at the sides of the mycelium. It contains a small num-

ber of spirals, tendrils, and blunt-ended spindles. On nutrient medium it produces very fluffy and pure-white colonies which have a smooth and dome-like surface. Quite frequently there is a powdery plaster-like film; the fluff may be yellowish or pinkish in colour.

The causative agent of epidermophytosis, *Epidermophyton inguinale* (R. Sabouraud, 1910), possesses a septate mycelium (Fig. 141) and a large number of spindles which occur in groups (5-7 spindles on a single hypha, in the form of a banana cluster). In cultures

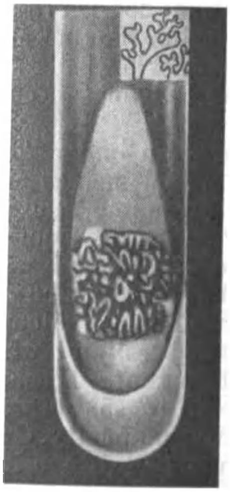


Fig. 138. *Achorion schoenleini* mycelium and colonies

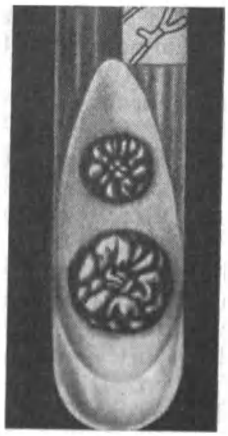


Fig. 139. *Trichophyton violaceum* mycelium and colonies

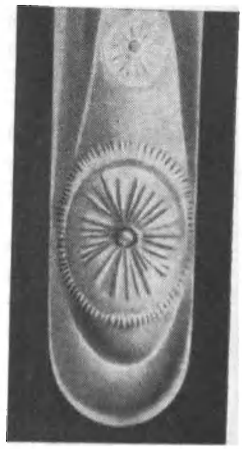


Fig. 140. Colonies of *Microsporum lanosum*

it produces velvet-like, powdery, folded and bulging colonies, yellowish-grey or, sometimes, green in colour.

Fermentative properties. The dermatophytes possess a variety of fermentative properties the majority of which are not stable. As a result of this, their biochemical properties cannot be used in laboratory diagnosis.

Toxin production. No soluble toxins are produced by the dermatophytes. The fungi contain an endotoxin and produce allergens which are responsible for increase of body sensitivity, particularly in the skin.

The antigenic structure has been insufficiently studied. The dermatophytes are devoid of species- and type-specific antigenic properties. Group serological reactions are revealed frequently by the complement-fixation reaction.

Resistance. The dermatophytes survive in infected hair for 4 years and in scales for more than 6 months. They are relatively resistant to exposure to heat and chemicals. Boiling inactivates the fungi in 15-30 minutes.

Pathogenicity for animals. Anthropophilic and zoophilic dermatophytes can be differentiated. The former affect only human beings (*Trichophyton violaceum*, *Microsporum ferrugineum*, *Achorion schoenleini*). The zoophilic dermatophytes are parasitic both on human beings and animals (*Trichophyton gypseum* is parasitic on man, sheep, rats, and mice; *Microsporum lanosum* is parasitic on man, cats, and dogs; *Trichophyton faviforme* is parasitic on bovine and small cattle). Dermatomycosis prevails in young animals, adult animals being affected less frequently.

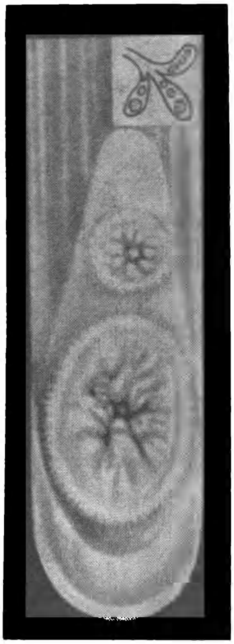


Fig. 141. *Epidermophyton inguinale* mycelium and colonies

Pathogenesis and diseases in man. The sources of infection are human beings and animals (cats, dogs, horses, cattle, etc.) suffering from the disease. The disease is acquired during direct contact of patients with healthy individuals in the family and in public establishments (hairdressing saloons, baths, swimming pools, laundries) and when articles (towels, clothes, hats, hair brushes, combs, sport equipment, etc.) are shared with the patient. Diseases occur sporadically. Outbreaks may occur in a community with a low sanitary level.

In human beings favus affects the hair, the skin of the scalp (accompanied by the loss of hair), and nails. The infected hair becomes grey and loses its luster and elasticity. Yellow-coloured scutula appear on the scalp. The scutula fuse and form an entire crust which frequently emits a mouse odour. Nail infection begins from their free ends with the appearance of yellow spots. The nails become lusterless, thickened, and brittle. They easily separate in layers and crumble.

Trichophytosis is characterized by infection of the scalp, the skin of the hands, and the nails. The hair breaks off at the surface of the skin, leaving hair stubs in the follicles and giving the appearance of badly cut hair or black dots. The fungus is found both inside and outside the hair. Pinkish-red squamous spots appear on the skin.

Microsporosis involves the hair, skin, and, less frequently, the nails. Lichen-like foci are formed on the skin and the hair breaks

off and is covered with whitish sheaths. The fungus penetrates into the hair and distributes along its full length.

Epidermophytosis is manifested by lesions in the skin, foot, hand, and nails. The hair is not infected. The development of eczematous foci and thickening and splitting of the nails are accompanied by skin inflammation. A secondary eruption (epidermophytid) is of an allergic origin.

The dermatophytes also embrace the causative agents of furfuraceous lichen—*Microsporon furfur* (C. Robeun, 1853) and erythrasma—*Microsporon minutissimus* (Burchard, 1859), and others.

The dermatophytes may also be responsible for a general infection of the body, producing a skin eruption and fever. Generalized skin eruptions are known as favids, trichophytids, and microsporids. In a number of cases fungi were isolated from the blood. The eruption is similar to that in scarlet fever in appearance.

Immunity. Particular importance is attributed to the state of body reactivity in dermatomycoses. Allergy cannot be considered to be an immune defense reaction, and the antibodies are not strictly specific. Immunity following infection is not of high grade and is relative.

Laboratory diagnosis involves the following methods:

1. Microscopic examination. The infected hair or scale is placed into a drop of a 10-20 per cent potassium hydroxide or sodium hydroxide solution on a glass slide. The preparation is heated slightly until steam appears and then covered with a cover slip and examined under the microscope at low magnification.

In favus the fungi can be seen as separate mycelial threads 3 to 5 μ in cross section. The segments are rectangular in shape. Air bubbles and drops of fat appear in the thick of the hair (Fig. 142, *3*). Mycelia and chains of spores are clearly observable in the scales from the skin and nails.

In trichophytosis the fungal segments, present in the infected hair, are 4-5 μ in size and occur in chains completely filling up the hair bulb (Fig. 142, *2*). Mycelial threads are clearly observable in the scales from the skin and nails. They are often convoluted and branched and septate. The scales from the nail contain clusters of circular spores.

In microsporosis the hair is surrounded by a sheath or muff consisting of circular spores which are 2-3 μ in size and form a mosaic pattern (Fig. 142, *1*). Septate dichotomously branching mycelial threads are seen clearly within the hair.

2. The pure culture is isolated by inoculation into Sabouraud's medium. To free the material under test from contaminating microflora, it is previously treated for several minutes with 2 per cent antiformin, 2-4 per cent formalin, or 2 per cent phenol. After this it is washed with sterile distilled water and inoculated. A simpler method for eliminating foreign flora is to cut the hair into very

small pieces with tempered dissecting needles and place the pieces for several minutes on a heated slide.

Additional methods include the agglutination and complement-fixation reactions and intracutaneous allergic tests with fungal extracts (favin, trichophytin, and microsporin). The intracutaneous tests are not stictly specific.

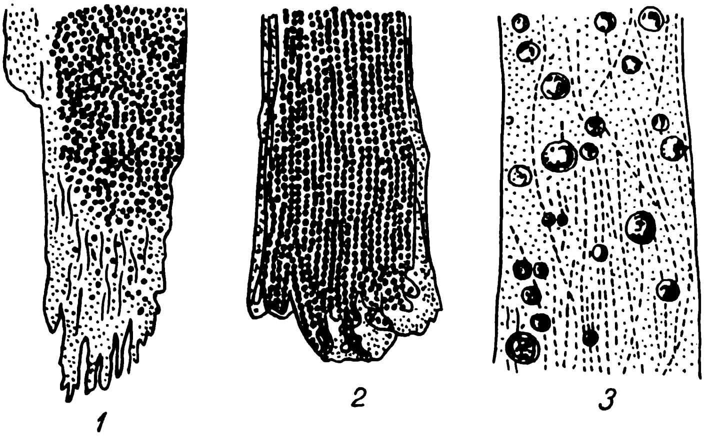


Fig. 142. Dermatophytes

1—Microsporum; *2—Trichophyton*; *3—Achorion* in infected hair

Treatment is accomplished with iodides, X-rays (roentgenoepilation), antiphlogistic warm baths, 3 per cent boric acid lotions, and desensitizing agents. At present the antibiotic griseofulvin is effectively used in the treatment of dermatomycoses.

Prophylaxis is ensured by thorough treatment of patients, medical supervision of sanitary-hygienic conditions in hairdressing saloons, baths, swimming pools, and sports grounds. Preventive measures also include veterinary control of animals, timely recognition, isolation, and treatment of human beings and animals affected with the disease, and systematic health education of the population.

PATHOGENIC PROTOZOA

The pathogenic protozoa include the aetiological agents of trypanosomiasis, leishmaniasis, trichomoniasis, lambliasis, toxoplasmosis, amoebiasis, malaria, and balantidiasis. The general characteristics and classification are given on p. 61. Due to the lack of accurate data, no information on the fermentative properties and toxin formation is given. The vegetative forms are not highly resistant. In the external environment pathogenic protozoa die relatively quickly. The *Entamoeba*, *Lamblia*, and *Balantidium* cysts survive for a long period of time, retaining their biological properties.

TRYPANOSOMES

The causative agents of African trypanosomiasis are two closely related microorganisms: *Trypanosoma gambiense*, discovered in 1902 by J. Dutton, and *Trypanosoma rhodesiense*, described by G. Fantham in 1910. They belong to the class of flagellates *Mastigophora s. Flagellata*, family *Trypanosomidae*, genus *Trypanosoma*.

Morphology. *Trypanosoma gambiense* can be seen as spindle-shaped cells with an undulatory membrane and pointed flagella at the ends (Fig. 143, *VI*). The organisms are motile, 25-40 μ in length and 1.4-20 μ in breadth. Their cytoplasm stains light-blue with the Romanowsky-Giemsa stain, while the nucleus, blepharoplast, and flagellum stain red.

The organisms are cultivated on a medium containing Ringer's solution and citrated human blood or on agar containing defibrinated blood (NNN or Nicolle, Novy, MacNeal medium).

Pathogenicity for animals. Some strains are pathogenic for the majority of domestic and laboratory animals. The causative agents multiply in the blood, internal organs, and bone marrow.

Pathogenesis and disease in man. Trypanosomiasis is a transmissible disease and is conveyed by the bite of the tsetse flies. In-

fection with trypanosomes gives rise to African sleeping sickness which is characterized by cachexia, anaemia, fever, oedema of the brain, chronic leptomeningitis, haemorrhages, and kidney lesions. The disease becomes chronic and persists for months and even years. There are alternating periods of fever and apparent recovery. This is followed by depression and progressive lethargy. The patient becomes more somnolent and sinks into a coma. A slight number of parasites are present in the blood. The trypanosomes are harboured in the tissues, particularly in the muscles, and in the cerebrospinal fluid. Quite frequently the patient dies from the disease. It has been known on the west coast of Africa since 1800. The disease does not occur in the USSR since its vectors are not among the country's fauna.

Immunity. Trypanosomiasis is accompanied by the formation of antibodies in the blood—trypanolysins, thrombocytobarins (Rieckenberg-Brusin phenomenon), complement-fixing antibodies, etc. The role of phagocytosis must not be neglected. Immunity is of low grade, insufficiently specific, and does not last for a long time.

Laboratory diagnosis comprises: (1) microscopic examination of blood by the thick film method (during the fever period) and of material obtained by puncture of the enlarged lymph nodes; (2) examination of the cerebrospinal fluid during the stage of lethargy. This method reveals an increase in the protein content and in the number of lymphocytes and, sometimes, the presence of trypanosomes.

Treatment is accomplished with antrypol (germanin) and pentamidine-isethionate (an aromatic diamine compound). In the second period of the disease patients are treated with intravenous injections of arsenic preparations (tryparsamide, arsobal) given in two or three courses.

Prophylaxis is ensured by a complex of measures which include recognition and treatment of patients, protection of the population from the bites of tsetse flies (*Glossina palpalis*), the use of insect repellents (dimethylphthalate, etc.), extermination of vector flies. Chemoprophylaxis is carried out by injecting healthy people with antrypol.

Trypanosomes infect cattle and wild herbivores which serve as reservoirs of the causative agent.

The causative agent of American trypanosomiasis (*Schizotrypanum cruzi*) was discovered in 1909 by C. Chagas in Brazil. Children are most susceptible to this disease. The infection is characterized by natural nidality and is conveyed by the bite of various bug species of the family *Triatomidae*. The natural reservoirs of the disease are wild animals—armadilloes, opossums, rodents, and monkeys.

Children younger than one year of age suffering from the disease very frequently die within a few days. In older children the condi-

tion acquires a subacute course. In adults the disease is accompanied by a rise of temperature, oedema of the face, and enlargement of the thyroid gland, lymph nodes, spleen, and liver. Infection produces no manifest clinical disease in the majority of adult cases. Chronic forms are accompanied by a disturbance of internal secretion, i.e., myxoedema, bronze coloration of the skin, and infantilism.

The trypanosomes may be detected in the peripheral blood, but they multiply not in the blood but in the tissues of the striated and cardiac muscles and in the central nervous system.

Laboratory diagnosis is made by: (1) examination of patient's blood in smears or thick films during the acute period; (2) guinea pig inoculation with 5-10 ml of patient's blood (various domestic and wild animals are readily infected with trypanosomes).

Treatment is accomplished with injections of a quinoline preparation Bayer 7602 and arseno-benzolsulphate Bayer 9736.

Prophylaxis is achieved by extermination of bugs, the vectors of the causative agent, with DDT preparations, etc. Chemoprophylaxis with pentamidine-isethionate preparations is carried out in endemic areas.

LEISHMANIA

The *Leishmania* parasite is a flagellate of the family *Trypanosomidae*. The causative agent of cutaneous leishmaniasis was discovered in 1898 by P. Borovsky when studying Penjdeh sore in Turkestan. W. Leishman and C. Donovan described the causative agent of kala-azar, the black fever in India, in 1903.

Two main forms of leishmaniasis can be distinguished: (a) cutaneous leishmaniasis and (b) leishmaniasis of the internal organs (visceral kala-azar).

CAUSATIVE AGENT OF CUTANEOUS LEISHMANIASIS

Morphology. In infected tissues (cells) Leishmania parasites (*Leishmania tropica*) occur as spherical or oval organisms. The tissue forms are nonmotile. The body surface is covered with a thin membrane. The organisms are 2-6 μ in length and 2-3 μ in breadth (Fig. 143, *IV*). The cytoplasm contains one or several vacuoles, a large spherical or oval-shaped nucleus, and a small nucleus (blepharoplast or kinetoplast) which is a tiny rod-like or rounded body bearing the remains of an extending flagellum (remnant). The Romanowsky-Giemsa method stains the cytoplasm light-blue, the nucleus bright-red, and the blepharoplast dark-red. Leptomonad (flagellate) forms (Fig. 143, *V*) are produced in the body of invertebrates (sandflies) and in cultures, the parasites grow up to 20 μ in length and up to 3 μ in breadth.

Cultivation. Leishmania organisms are grown on agar containing defibrinated rabbit blood (NNN medium). On this medium they multiply at 18-22°C and are arranged in the form of a rosette.

Pathogenicity for animals. The gerbil, suslik, and other desert rodents are sources of infection and reservoirs of the causative agent of cutaneous rural leishmaniasis. American cutaneous leishmaniasis is characterized by natural nidality and is spread among wild animals. The reservoir of the aetiological agent of visceral leishmaniasis is human beings, dogs, and cats; human beings suffering from manifest and asymptomatic forms of the disease and animals infected with Leishmania parasites serve as sources of the infection. During laboratory infection, which is not always successful, pathogenic Leishmania organisms affect monkeys, dogs, mice, hamsters, susliks, gerbils, squirrels, cats, rabbits, and guinea pigs. The parasites produce a generalized infection and are found to be present in the spleen, liver, and bone marrow.

Pathogenesis and diseases in man. Two forms of cutaneous leishmaniasis are encountered in the USSR (in Central Asia and Transcaucasia).

The dry form (Ashkhabad sore, late ulcerating leishmaniasis) is caused by *Leishmania tropica var. minor*.

The incubation period is of long duration (up to 5 months and longer). A copper-coloured spot appears on the skin of the face, neck, hands, and legs. The spot gradually develops into a tubercle which grows, and necrotized tissue forms in its centre. The hard skin tubercle becomes covered with a brown-red scab. When the latter is removed, an ulcerated surface covered with loose granulation tissue containing a large number of Leishmania organisms is revealed. The disease lasts about a year, and is known by the Russian name of "godovik" ("yearling").

The source and reservoir of the dry-form or anthroponose leishmaniasis (urban) are sick human beings and domestic dogs. Infection is conveyed by the sandfly vector of the genus *Phlebotomus*.

The moist form (Penjdeh sore, acute necrotizing leishmaniasis) is produced by *Leishmania tropica var. major*. The condition is characterized by a relatively short incubation period (from 1-2 weeks to $1^1/_2$-2 months). The papules are oedematous and the ulcer edges are loose and with an uneven outline. The leishmaniomas are surrounded by tubercles which develop as a result of dissemination of the causative agent. Cicatrization takes place in 3-6 months. Penetrating into the lymphatics, the Leishmania organisms produce limphangitis and, less frequently, regional lymphadenitis.

The source and reservoir of the causative agent of the moist-form or zoonotic (rural) cutaneous leishmaniasis are wild rodents which live in burrows (gerbils, susliks). The disease occurs in the Turkmen and Uzbek SSR. The vectors of the disease are the *Phlebotomus* sandflies.

Immunity. The disease leaves immunity lasting for several years. Individuals who had suffered from cutaneous leishmaniasis of the rural type become insusceptible to the urban type of leishmaniasis, and this testifies that these two types which produce cross immunity are related in antigenic structure. This was taken into account in specific prophylaxis of cutaneous leishmaniasis. The vaccine is composed of a live culture of the rural type of Leishmania organisms, and is injected intracutaneously in a single dose.

Laboratory diagnosis comprises (1) detection of the Leishmania parasites in granulation tissue; (2) inoculation into agar containing defibrinated blood.

Treatment. Acrichine (quinacrine) is injected deep into the papule. In addition, disinfectant ointments are used (protargol, rivanol). Cases with ulcerated leishmaniomas are given antibacterial agents for the suppression of secondary infection.

Prophylaxis. Cutaneous leishmaniasis control is effected with general measures (early diagnosis. extermination of sandflies and dogs and rodents infected with leishmaniasis, and vaccination after Latyshev). On arrival at a leishmaniasis foci individuals are injected live *Leishmania* culture into the upper part of the upper arm or thigh. Immunization produces a stable immunity.

Mucocutaneous leishmaniasis (espundia) is a variety of cutaneous leishmaniasis. It is characterized by natural nidality and occurs in Mexico, Central America, and in all countries of South America (with the exception of Chile). The disease is caused by *L. brasiliensis.* The vector is the sandfly *Phlebotomus intermedius*. Another variety is the Sudan or nodular leishmaniasis caused by *L. nilotica.* Treatment is accomplished with antimony preparations. Antibiotics and sulphonamides are prescribed in the presence of bacterial complications. Prophylaxis is achieved by measures aimed at extermination of sandflies and protection from them. Immunization is not carried out.

CAUSATIVE AGENT OF VISCERAL LEISHMANIASIS

The disease (kala-azar) is caused by *Leishmania donovani.* The incubation period varies from several weeks to 2-3 months and is followed by fever and long-term remissions. The temperature is moderately elevated, the spleen and liver become enlarged, and progressive anaemia develops. Intestinal ulcers, lesions in the bronchi and lungs, oedema and haemorrhages in the internal organs, mucous membranes, and skin may also occur. The skin turns a dark colour (therefore the disease is known as black fever). Kala-azar is characterized by a chronic course and lasts for many years. Death rate ranges from 70 to 80 per cent.

The reservoirs of infection are human beings and domestic animals (dogs, cats). The source of infection is patients with manifest

and latent forms of leishmaniasis and domestic animals infected with Leishmania organisms. Visceral leishmaniasis is also characterized by natural nidality. It occurs in India, China, and Central Africa where it is known as kala-azar. In the USSR visceral leishmaniasis is registered in Central Asia and Transcaucasia. The infection is transmitted by the bite of a sandfly.

C. Nicolle described a children's disease in Tunis which is similar to kala-azar. He designated the causative agent *L. infantum* and it is probably a variety of *L. donovani*.

The disease leaves quite a lasting immunity. No reinfections have been noted.

Laboratory diagnosis comprises the following: (1) examination of sternal bone marrow obtained by sternal puncture; puncture of the liver and lymph nodes is performed in some cases. Smears are stained by the Romanowsky-Giemsa method; (2) the material obtained by puncture is inoculated onto agar containing defibrinated blood.

Examination of smears is the most accessible and valuable method.

Treatment is accomplished with solusurmine and neostibosan.

Prophylaxis comprises the following measures: early diagnosis, opportune treatment, rodent control, and extermination of sandflies and of dogs infected with leishmaniasis.

LAMBLIA AND TRICHOMONADS

CAUSATIVE AGENTS OF LAMBLIOSIS

Lamblia intestinalis, discovered in 1859 by W. Lambl, belongs to the flagellate class, family *Hexamitidae*.

Morphology. Lamblias are bilateral, symmetrical, pear-shaped organisms with an elongated posterior end and two symmetrically placed nuclei. The body of the parasite is from 10 to 18μ long and from 8 to 10μ broad. It has a disk-like depression, a peculiar sucker at the blunt end by means of which the parasite fastens itself to the surface of the intestinal epithelium. Two supporting filaments run along the middle line of the body. The organism moves by means of four pairs of flagella (Fig. 144). The parasites form oval-shaped cysts which are 10-14μ in length and 7.5-9μ in breadth and have four nuclei.

Cultivation. For over a hundred years efforts to grow a *Lamblia* culture were unsuccessful. At present lamblias are cultivated on media containing extracts of yeast-like fungi, in particular *Candida*.

Pathogenicity for animals. Lamblias are found in synanthropic rodents, dogs, cats, sheep, goats, horses, cattle, etc. However, these organisms are not identical to lamblias in human beings.

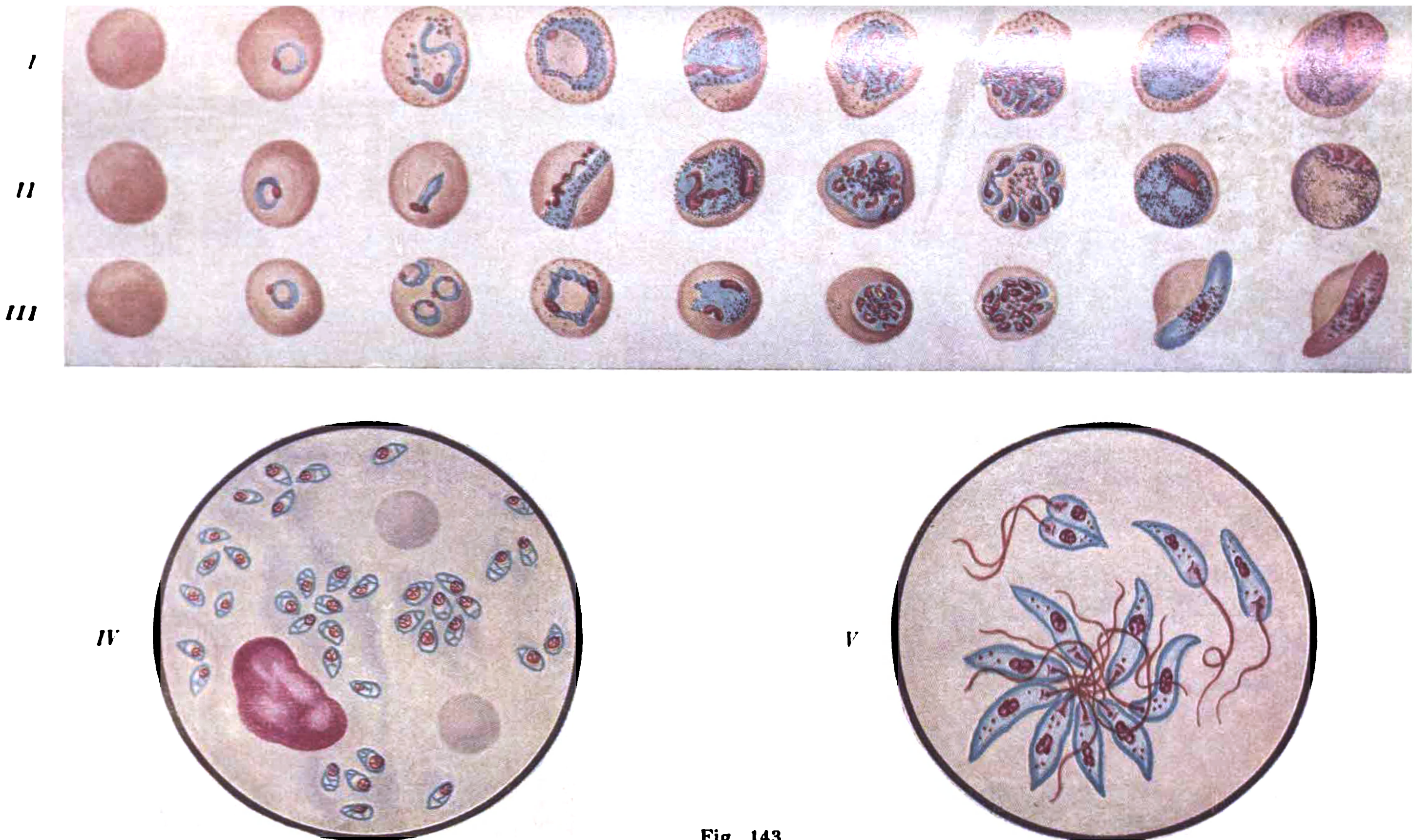


Fig. 143.

I—developmental stage of *Plasmodium vivax*; *II*—developmental stage of *Plasmodium malariae*; *III*—developmental stage of *Plasmodium falciparum*: *IV*—*Leishmania* in organs: *V*—*Leishmania* in culture; *VI*—*Trypanosomes* (see over)

IV

Pathogenesis and disease in man. The source of infection is a human being who discharges cysts with the faeces. The chitin membrane of the cyst dissolves on entering the intestines. The vegetative forms of lamblias multiply in the small intestine and penetrate into the duodenum and gall-bladder. Inflammatory processes in the mucous membrane, which occur in dysentery and helminth contamination, and the presence of fungi are conducive to lamblia development. The liver and intestines are involved (chronic duodenitis, enterocolitis). Dyspeptic symptoms (nausea, pyrosis, hypoacidity)

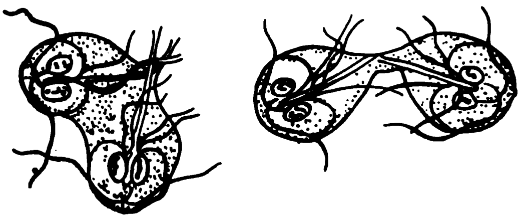


Fig. 144. Lamblia at stage of multiplication

and general emaciation are noted, and cholecystitis and hepatitis develop quite frequently.

Immunity has not been studied. There are no data in regard to postinfection immunity.

Laboratory diagnosis is made by microscopic examination of the duodenal contents or of faeces.

Treatment is accomplished with acrichine (quinacrine) given orally or used in a solution for irrigating the duodenum. Mixed infection calls for complex treatment with due consideration of the character of the parasitocoenosis co-members (vermifuge treatment, antidysentery drugs).

In view of a new co-member of the parasitocoenosis being revealed (fungal flora), antifungal preparations (nystatin, etc.) are now in practice along with acrichine. These preparations are destructive to the fungi and thus create unfavourable conditions for lamblias. Antibiotics (penicillin, chlortetracycline, etc.) are of no therapeutic value and quite frequently they facilitate lamblia development.

Prophylactic measures are similar to those carried out in prevention of intestinal infection.

PATHOGENIC TRICHOMONADS

Three species of tetraflagellate trichomonads belonging to the class of flagellates, family *Trichomonadidae*, are parasitic within the human body.

Trichomonas vaginalis was discovered in 1836 by A. Donne. It varies from 20 to 36μ in size and has an undulating membrane connected to a thin fibril. This *Trichomonas* species inhabits the lower parts of the genital apparatus and is most highly developed in patients from 18 to 45 years of age.

Trichomonas intestinalis was discovered in 1860 by C. Davainea. The parasite ranges from 10 to 17μ in size. It has a very long coiled undulating membrane (Fig. 145) which extends far beyond the parasite's body in the form of a trailing flagellum. It is found in the large intestine of human beings.

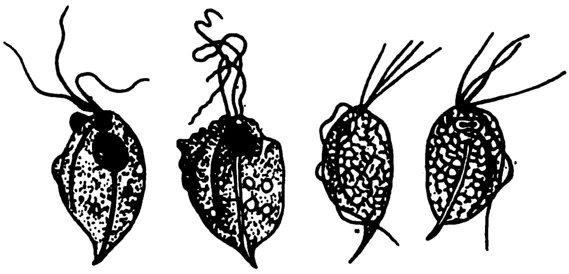


Fig. 145. *Trichomonas intestinalis*

Trichomonas buccalis was discovered in 1773 by O. Muller. The undulating membrane is short and the end fibril is hardly discernible. The parasite is from 10 to 17μ in size. It is found mainly in the mouth of elderly persons with bad teeth, periodontosis, etc.

In males infection with *Trichomonas* parasites occurs in the form of acute, subacute, or chronic urethritis. It has been ascertained that males may convey the *Trichomonas* organisms to healthy females.

As the infectious disease develops, lactobacilli of Dederlaine disappear from the vaginal microflora while staphylococci, streptococci, enterococci, *E. coli*, some anaerobes, vaginal vibrios, spirochaetes, corynebacteria, etc., persist and multiply. This symbiosis of trichomonads and bacteria results in the development of a pathological condition.

Infection is transmitted by the genital route and through toilet articles, sponges, and lavatory pans and stools. The intestinal form of trichomoniasis is spread by the intestinal-faecal route. The source of infection and reservoir of the causative agent are patients and trichomonad carriers.

For laboratory diagnosis two smears each are made of discharge from the vagina, cervix uteri, and urethra; one of the smears is stained with the Romanowsky-Giemsa stain, and the other with the Gram stain. The whole bacterial biocoenosis is demonstrable

in the stained smears. Native preparations are examined for motility. Trichomonads are cultivated by inoculation into meat-peptone broth (pH 6.0) containing 0.1 per cent glucose, 10 per cent human or horse serum, 300 units of penicillin, and 200 units of streptomycin per 1 ml of medium. The culture is incubated at 36°C for 3 to 6 days and then the growth is examined for the presence of *Trichomonas* parasites.

Treatment is accomplished with trichomonacide, osarsol (acetarsone), aminarsone (carbasone), yatren, sulphazine, and boric acid. Trichomonal urethritis in males is treated with carbarsone or triflocide.

Prophylaxis measures are the same as in venereal diseases.

PATHOGENIC TOXOPLASMS

The causative agent of toxoplasmosis, *Toxoplasma gondii*, was isolated from *Ctenodactilis gondii* in Algieria in 1908 by C. Nicolle and L. Manseux. In the years following this parasite was found to be present in many species of domestic and wild animals. It belongs to the class *Flagellata* and resembles the *Leishmania* organisms.

Morphology. *Toxoplasma gondii* may be crescent-, pear-, or oval-shaped. Its ends may sometimes be pointed, and it varies from 4 to 7μ in length and from 2 to 4μ in breadth, but larger forms, 10-12μ long and 5μ broad, may also occur (Fig. 146). Electron microscopy disclosed the presence of very thin fibrillas on the parasite's body. Toxoplasms are motile and are capable of gliding and rotatory movements, although no flagellas have been observed.

Toxoplasms stain readily by the Romanowsky-Giemsa method, the cytoplasm taking up a light-blue colour and the nucleus a ruby-red colour. They multiply by longitudinal fission. The parasite may occur freely in the host or within various cells of the histiophagocytic system, nerve tissue, liver, placenta, etc. Young forms (pseudocysts) accumulate in the cells during the process of multiplication, invade the cell cytoplasm, and are subsequently destroyed. Toxoplasms are not parasitic within the erythrocytes and schizogony has not been exhibited; however, this problem has not been completely elucidated.

Fig. 146. Toxoplasms phagocytized by a cell

Cultivation. Toxoplasms are grown in developing chick embryos. Usually a drop of peritoneal exudate obtained from mice infected with toxoplasms is introduced into the embryo. The infected embryos die on the fifth or sixth day. The toxoplasms may also be cultivated on special tissue cultures. Good growth is obtained in shaken tubes which contain tissues of mouse and human embryos. The laboratory animals used for cultivating toxoplasms are guinea pigs, mice, rabbits, susliks, and hamsters.

Resistance. Toxoplasma organisms poorly resist exposure to environmental factors (high temperature, desiccation, irradiation) and disinfectants. They survive for a long time only in the bodies of domestic and wild animals and in arthropods. At low temperatures they remain alive for a period of several minutes to several days. and heating to a temperature of 45-60°C kills them in 5-15 minutes.

Pathogenicity for animals. Toxoplasms are pathogenic for numerous domestic and wild animals and birds. The parasites have been found in dogs, cats, swine, sheep, rabbits, guinea pigs, hares, susliks, rats, mice, field voles, monkeys, and in a number of birds (pigeon, chicken, wood-grouse, black grouse). It is assumed that the trombiculid mite may serve as the reservoir of pathogenic toxoplasms. The disease in domestic animals is expressed by fever, respiration disturbances, diarrhoea, lesions in the central nervous system, premature delivery, abortion, and barrenness. Post-mortem examination of diseased animals reveals pneumonia, intestinal ulcers, necrotic foci in the liver, enlargement of lymph nodes, and exudates.

Pathogenesis and diseases in man. Toxoplasmosis is a widespread disease and occurs in all countries. Man acquires the infection from dogs, particularly from those bred in kennels, and from cats, sheep, and other animals.

Infection takes place through the alimentary and respiratory tracts. The causative agent gains entrance by the mucous membranes (conjunctiva, vagina, mouth). The parasite may also enter the body with food and water which have been insufficiently heat-treated, and may be transmitted by the bites of arthropods (ticks, body louse). A person with toxoplasmosis is of no importance in the spread of the disease among human beings, with the exception of infection of the foetus by the mother through the placenta.

Toxoplasmosis may be congenital or acquired. Congenital toxoplasmosis is characterized by hydro- or microcephalus, cerebral calcification, lesions in the organs of vision (chorioretinitis), cirrhosis of the liver and enlargement of the spleen. Pneumonia, enterocolitis, nephritis, hepatitis, and a high (39-40°C) or subfebrile temperature are also typical manifestations. Irreversible pathologic conditions remain in the central nervous system, internal organs, and skeleton in children who survive the disease. These conditions are responsible for profound physical and psychic disorders which

may give rise to oligophrenia, schizophrenia, epilepsy, idiotism, etc. Asymptomatic toxoplasmosis in the mother leads to infection of the foetus and this results in abortion or birth of a dead child.

The alimentary route of infection is most frequent. It takes place on ingestion of meat, milk, and dairy products of animals sick with toxoplasmosis, uncooked eggs of affected birds, and water contaminated by sick animals.

In some cases toxoplasms may penetrate into an animal or human body through the bites of blood-sucking arthropods which serve as mechanical vectors of the organisms. Infection may also be transmitted via air-droplets and through injured skin and mucosa Infection of laboratory workers, obstetricians, gynaecologists, and surgeons who had come into contact with patient's blood or infective material has been described in literature.

Depending on the immunobiological condition of the infected individual and the number of parasites which invade the body the disease may acquire an asymptomatic, mild, or severe course. Re-infections give rise to sensibilization of the body and the development of specific allergy which persists for quite a long time after recovery.

Manifestations of toxoplasmosis in adults include maculo-papular eruption covering all parts of the body except the palms, soles, and scalp. Pneumonia, enterocolitis, nephritis, hepatitis, and high or subfebrile temperature are of quite frequent occurrence. A large number of patients display eye lesions (choroiditis, periphlebitis of retinal vessels, optic neuritis, etc.). The disease is characterized by a wide range of clinical forms. Latent forms are recognized only by means of serological reactions. Quite frequently infection becomes chronic, exhibiting allergy. Antibodies produced by the body do not afford sufficient protection against the parasite.

Laboratory diagnosis comprises the following:

1. Microscopic examination of fluids and organs of sick human beings and animals for the presence of toxoplasms. Smears are made from centrifuged deposits of cerebrospinal and pleural fluids and stained with the Romanowsky-Giemsa stain. In cases of pneumonia the sputum is examined, and in some cases microscopy of bone marrow and bioptic lymph nodes is performed. Fluids and tissues (liver, brain, spinal cord, spleen, lungs, etc.) are examined post mortem.

2. Biological tests for demonstrating toxoplasms are employed by intracerebral or intraperitoneal infection of susceptible animals (mice, white rats, guinea pigs, rabbits, hamsters, and pigeons) with blood, cerebrospinal fluid, ocular fluid, and tissue emulsions. As a rule, an acute disease develops in the infected animals, and mice die on the seventh or tenth day. Excessive exudate and a large number of toxoplasms accumulate in the abdominal cavity beginning from the third or fourth day. The parasites can be demon-

strated in the brain and various organs. If results are negative passage is repeated three or four times. Infected mice are placed under survey for 2 weeks and the guinea pigs, for 6 weeks.

3. The serological test of Sabin and Feldman is based on the phenomenon that toxoplasms present in patients' sera lose their ability to stain with methylene blue under the influence of antibodies. Toxoplasms obtained from the peritoneal exudate of mice on the third or fourth day after infection are added to the serum under test. The test tubes are placed in a water bath at 37-56°C for 30-60 minutes after which a methylene blue solution (pH 11.0) is added. The cytoplasm of the parasite will not stain if the serum contains specific antibodies, while in the control test tubes containing serum of healthy individuals the toxoplasms will turn blue. The reaction is valued positive in a 1:4-1:64 dilution of the serum if more than 50 per cent of the parasites remain unstained.

4. The complement-fixation reaction is performed by the usual method. Toxoplasms isolated from chick or duck embryos or from the peritoneal fluid of infected mice serve as antigens. The reaction is usually positive in the later period of the disease.

5. Procedures for an intracutaneous test are similar to those for the Mantoux tuberculin test. Results are checked in 48 hours. The erythema should not exceed 10×10 mm in size. The skin test is not performed in children younger than 2 years of age, in weakened individuals with flaccid skin, and in old people since in these cases it often yields a negative result. The haemagglutination reaction using ram erythrocytes previously sensitized with the toxoplasm antigen (by adsorbing them) and the patient's serum is also employed

Treatment. Sulphonamides (sulphadimezin) and chloridine which is identical with daraprim are used. It is recommended to prescribe sulphonamides together with chloridine according to instructions for treatment of toxoplasmosis.

Prophylaxis. The complex of preventive measures should be carried out jointly with the veterinary service. The occurrence of toxoplasmosis in areas with natural nidality necessitates destruction of wild animals, recognition of cases and carriers of the disease among domestic animals and their isolation and treatment. For laboratory confirmation the following methods are used: complement-fixation reaction, allergy test, and isolation of the causative agent from the internal organs, lymph nodes, and other pathological material. Stray dogs and cats are most dangerous and must be destroyed.

The meat of animals suspected of having toxoplasmosis must be thoroughly heat-treated, milk must be boiled, and eggs must be cooked for 5 minutes. Systematic rat extermination is carried out in localities with cases of toxoplasmosis, cadavers of domestic and wild animals which had died from toxoplasmosis are flooded with

kerosene or other disinfectants and buried not less than 1.5 m deep in special graves.

Health education on personal hygiene is taught among the population (washing of hands before meals and after handling animals and animal products, and the prohibition of preparing food from insufficiently cooked meat products, in particular liver).

Special attention should be given to prevention of occupational infection of workers in the veterinary service, slaughter houses, cattle farms, refuse-processing establishments, and laboratories and among dairy maids and hunters of game animals. All workers of the above professions must be examined periodically and treated if found to be affected with the disease.

All women with a history of spontaneous abortion, premature labour, and those who had given birth to dead or deformed children must be examined by laboratory methods for prevention of congenital toxoplasmosis. Recognized cases must be given a complete course of treatment.

CAUSATIVE AGENT OF AMOEBIASIS

The causative agent of amoebiasis, *Entamoeba histolytica*, was discovered in 1875 by F. Loesch. In 1903 the organism was studied in detail by F. Schaudinn who differentiated two amoeba species, *Entamoeba histolytica* and *Entamoeba coli*, which belong to the class *Sarcodina*, family *Entamoebidae*.

Morphology. *Entamoeba histolytica* occurs in the human body in three forms:

(1) vegetative large tissue form which feeds on the erythrocytes and does not become encysted—*Entamoeba histolytica forma magna*;

(2) vegetative small commensal encysted form which lives in the lumen of the large intestine—*Entamoeba histolytica forma minuta*;

(3) cysts which develop from the small forms.

Entamoeba histolytica penetrates into the tissues of the large intestine under the influence of a number of factors (lowered resistance of the human body due to various diseases, intoxications, overheating, overstrain, injuries, and wounds) and gives rise to deep changes there. It produces proteolytic substances which cause lysis of cells and tissues. The parasite grows to a size of 30-50 μ (23 μ on the average) and becomes capable of phagocytizing erythrocytes. This vegetative form is known as the tissue form, *Entamoeba histolytica forma magna*. It is usually found in the mucosanguineous stool of an amoebiasis patient. The organism's ectoplasm is translucent, while its endoplasm is granular.

Entamoeba histolytica forma minuta is the main form of the causative agent of amoebiasis. Its size ranges from 12 to 25 μ. The nucleus is spherical in shape and is from 3 to 7 μ in diameter. The chro-

matin is distributed evenly and close to the nucleus membrane in the form of small granules. Phagocytized bacteria are found in small numbers in the endoplasm. The ectoplasm is poorly developed and accumulates in the pseudopodia, motility is decreased. Amoebas inhabit the upper part of the large intestine of a healthy human being and are known as luminal, cavity or commensal forms. The luminal form enters the lower part of the large intestine with the faecal masses. Here the conditions are unfavourable for the vegetative form (the pH of the medium changes as a result of dehydration and putrefactive processes). The luminal form at first transforms into the precystic stage and later into a cyst.

The cysts are spherical in shape with a diameter of 8 to 16 μ. They have thin-walled, double membranes. Mature cysts contain four nuclei similar in structure to those of the vegetative forms. Immature cysts have one, two, and, sometimes, three nuclei. Cysts are discharged with the faeces for a long period of time and sometimes throughout life. They may re-enter the human body with foodstuffs or water and transform into luminal forms in the intestine. At this stage the developmental life cycle of the *Entamoeba histolytica* is completed.

Nonpathogenic amoebas, *Entamoeba coli*, also live in the intestines of human beings. They are somewhat larger than *Entamoeba histolytica*. Their cytoplasm is granular and the vacuoli contain bacteria, leucocytes, food particles, and glycogen, but no erythrocytes. Pseudopodia seldom occur, the cysts are larger than those of *Entamoeba histolytica* and possess 8 nuclei (Fig. 147).

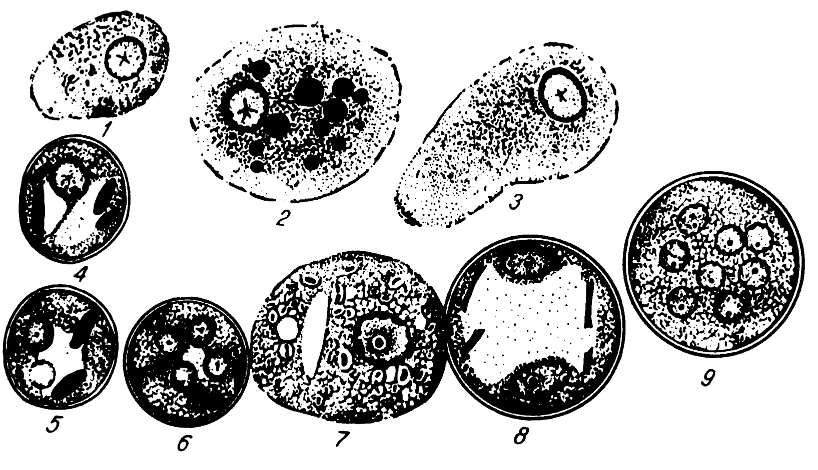


Fig. 147. *Entamoeba histolytica* (*1-6*) and *Entamoeba coli* (*7-9*)

1—luminal-commensal form; *2*—tissue form containing phagocytized erythrocytes; *3*—tissue form without erythrocytes; *4*, *5*, and *6*—one-, two-, and four-nuclear cysts; *7*—vegetative form; *8* and *9*—two- and eight-nuclear cysts

Cultivation. The causative agent of amoebiasis is grown on the Pavlova medium (500 ml distilled water, 4.25 g NaCl, 0.3 g Na_2HPO_4, 0.5 g KH_2PO_4; the mixture is dispensed into test tubes, in 9.5 ml amounts, and 0.5 ml of horse serum and one loopful of starch are added to each test tube).

Pathogenicity for animals. Kittens and dogs infected through the rectum display typical dysentery lesions in the rectum and abscesses in the liver (extraenteric form of amoebiasis). The white rat is one of the best animals for experimental amoebiasis.

Pathogenesis and disease in man. Infection is conveyed through cysts which occur in water and foodstuffs contaminated with faeces. Amoebiasis prevails in eastern regions where water is supplied from aryks and the sanitary standards are low. The disease is widespread in the Middle East, India, Indo-China, North and Central Africa, Indonesia, South America, etc.

The disease becomes chronic and is accompanied by lesions in the large intestine, particularly in the caecum and ascending colon. The patient's stool has the appearance of raspberry jelly and is uniformly impregnated with blood.

The parasite localizes in the colon and rectum in which inflammatory oedema, ulcerations, necrosis of adjacent tissues, and gangrenous decomposition develop. The amoebas penetrate the mucous, submucous, and muscular coats, invade the intestinal vessels, and reach the liver along the branches of vena porta.

Complications arise in the form of abscesses and necrosis of the liver and, sometimes, abscesses of the lungs and brain. Occasionally the amoebas may enter the greater circulation, and in such cases any of the organs may be involved.

Various species of bacteria play an essential role in the pathogenesis of amoebiasis, being responsible for the pathological process in combination with the entamoebas. Experimental studies of the parasitocoenosis have shown that some species of streptococci, pneumococci, and *E. coli* in association with *Entamoeba histolytica* produce more severe forms of the disease and cause a higher morbidity in laboratory animals.

The pathogenesis of amoebiasis is still not completely clear. It has been ascertained that if the intestinal mucosa and the body as a whole are normal, the *Entamoeba histolytica* may live in the intestinal lumen as a commensal causing no disease in the human being. A number of factors have been proven to facilitate penetration of the parasite into the tissues. Pseudopodia allow the *Entamoeba histolytica* to enter the tissues, while the proteolytic properties of the parasite (the ability to produce a cytolytic enzyme) render it capable of destroying the intestinal epithelium. In addition, the parasite produces a toxic substance which possesses cytolytic and complement-fixing properties.

Immunity. A relatively marked congenital insusceptibility to

amoebiasis exists among human beings. There is evidence for this in the fact that widespread cyst carriage (from 5 to 30 per cent) is not followed by disease. It is assumed that insusceptibility is associated with a condition of nonsterile immunity. Of great importance are the resistance of the intestinal wall tissues and the ability of the body to neutralize the harmful effect of the co-members of the parasitocoenosis. Immunity in amoebiasis is unstable.

Laboratory diagnosis. Fresh stools are examined under the microscope using a temperature-controlled microscope stage. *Entamoeba histolytica* can be demonstrated in the stools. For demonstrating cysts and glycogen granules under the microscope, a strong Lugol's solution is added to the faeces (1 g iodine, 2 g potassium iodide, and 20 ml distilled water).

The presence of pathogenic amoebas may also be revealed by histological examination of tissues (stained with methylene blue, safranine, haematoxylin-eosin, and iron haematoxylin).

Laboratory amoebiasis may be reproduced by infecting kittens and white rats. The resulting condition is typical of the disease.

Treatment. Emetine hydrochloride, aminarsone, yatren, chlortetracycline, oxytetracycline, gramicidin, resotren (a chemical compound consisting of chloroquine and iodohydroxyquinoline), and purimycin are used. A combination of antibiotics which are administered in capsules (polymyxin B, dihydrostreptomycin, neomycin, and bacitracin) and combined treatment with oxytetracycline and yatren are very effective. Antibacterial drugs—gramicidin, penicillin, synthomycin (chloramphenicol), and sulphonamides are given for the concomitant bacterial microflora which aggravate amoebiasis.

Prophylaxis. All patients are transferred to a hospital and adequately treated. Preventative measures against amoebiasis also include removal of all refuse, fly control, and protection of foodstuffs and water from flies and contamination with faeces. The staff of catering establishments must be examined for cyst carriage. Health education of the population, aimed at raising the cultural level, and introducing of hygienic habits are carried out.

PLASMODIUM MALARIAE

Plasmodium malariae belongs to the class *Sporozoa* (E. Metchnikoff, 1887), family *Plasmodidae*, genus *Plasmodium*. The organism is capable of infesting erythrocytes and other vertebrate cells.

In 1879 V. Afanasyev and K. Vinogradov voiced an opinion that malaria was caused by parasites ("heterotoxic") present in the blood. In 1880 A. Laveran discovered the causative agent of quartan malaria, and in 1890 J. Grassi and R. Feletti found the parasite responsible for tertian malaria. The aetiological agent of tropical

malaria was discovered in 1897 by W. Welch, and the causative agent of tertian malaria (*Plasmodium ovale*), in 1922 by J. Stevens.

The structure of the causative agents of malaria has been studied by the application of a special staining method elaborated by D. Romanowsky in 1891.

In 1895 R. Ross proved the role of mosquitoes in the epidemiology of avian malaria. The role of mosquitoes in the epidemiology of human malaria was demonstrated by P. Manson only in 1898, although the fact that these parasites transmitted malaria was known to the inhabitants of tropical Africa a long time ago.

Morphology of plasmodia. The merozoite is the youngest form of the parasite, appearing as the result of the splitting (merulation) of a mature schizont. It is spherical or oval and small in size (1-2 μ). The merozoites consist of cytoplasm and a nucleus and are not capable of amoeboid movements. The Romanowsky-Giemsa stain colours the cytoplasm light-blue and the nucleus red. Merozoites penetrate into the erythrocytes and give rise to asexual forms of the parasites. After the parasites disappear from the blood they may persist in the tissues. At the same time sexual forms, gametocytes (gamonts), are formed in the human erythrocytes, which develop further only in the mosquito body (sporogony).

Having gained entrance into the erythrocyte, the young schizont (ring-form stage) grows larger and a vacuole appears in its cytoplasm. At this stage the malarial parasite has irregular contours and resembles a ring with a ruby.

The semimature schizont is capable of amoeboid movement. As it grows, a pigment appears within it (a product of haemoglobin breakdown) in the form of dark-brown spots.

The mature schizont becomes rounded and pulls in its pseudopodia by the time of complete maturation, occupying almost the entire erythrocyte. Merulation takes place at this stage, i.e., the nucleus and cytoplasm divide forming from 6 to 24 merozoites, depending on the species of the parasite. The pigment accumulates in the centre in a compact clump. On the completion of merulation the erythrocytes are destroyed and the merozoites are released into the blood plasma. Some of them again penetrate into erythrocytes, while others (the majority) are killed by the immune factors of the body.

The gamonts are sex cells and are subdivived into female (macrogamonts) and male (microgamonts) cells. The macrogamonts are 12-14 μ in size and contain large pigmented granules. Their nuclei are small, compact, stain a red colour and occur along the cell edges. The microgamonts are smaller, their cytoplasm stains a paler colour, and the nuclei are large, slightly diffuse, and occur in the centre.

Development of the malarial plasmodia. Plasmodia undergo an asexual and sexual cycle of development (Fig. 148). The asexual

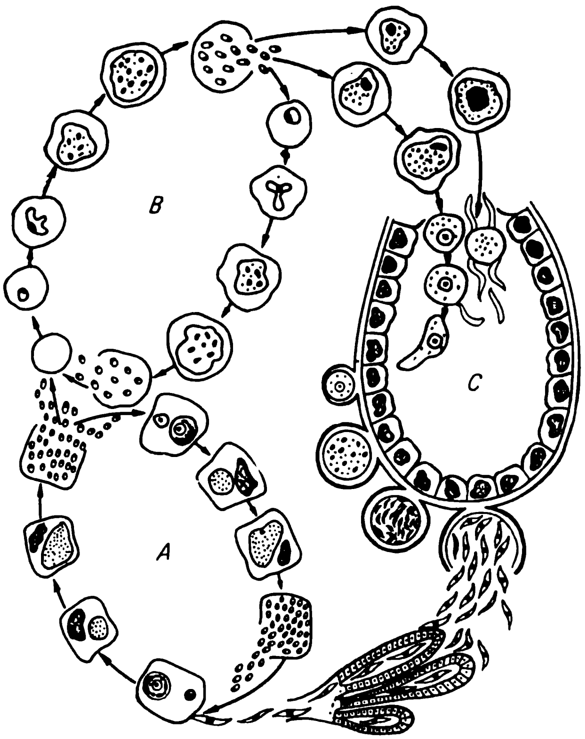


Fig. 148. Life cycle of malarial parasites

A—tissue (exoerythrocytic) schizogony; B—erythrocytic schizogony; C—sexual multiplication

cycle of the development of the malarial parasite takes place in the human body and is known as schizogony. When a human being is bitten by a female *Anopheles* mosquito infected with the causative agent of malaria, the malarial plasmodia enter the blood with the mosquito's saliva in the form of thread-like cells known as sporozoites and undergo a definite cycle of development. They infest the cells of the liver and of the other organs and tissues in the human body. This is the exoerythrocytic stage. In the liver cells the sporozoites become rounded and grow to a definite size. After this they divide forming a large number of merozoites. *Pl. falciparum* undergoes one cycle of tissue schizogony and *Pl. vivax*, two cycles. Then, merozoites capable of penetrating into the erythrocytes are formed. There are no clinical manifestations of the disease in infected individuals during the tissue developmental cycles of the parasites. Some of the merozoites enter the erythrocytes and trans-

form within them into amoeboid bodies which grow there until maturity. This is followed by the stage of division (merulation) resulting in the formation of merozoites which again penetrate into the erythrocytes and repeat the cycle of development.

At a certain stage the merozoites transform into the sexual forms, macro- and microgamonts. In the stomach of the mosquito the microgamonts fertilize the macrogamonts and as a result of this a mobile form, the ookinete, is produced. The latter penetrates into the stomach wall where it transforms into an oocyst.

The time needed for the development of the malarial plasmodium in the mosquito body depends on the species of the parasite and on the environmental temperature, but averages 7-9 days, although it sometimes ranges from 7 to 45 days. The mature oocyst may be up to 60μ in diameter and is filled with sporozoites. It bursts and the sporozoites are released into the mosquito body cavity. They accumulate in the cells of the salivary glands and are introduced into the human body through a bite. In the human body they first undergo exoerythrocytic schizogony in the tissues and then gain entrance into the erythrocytes where erythrocytic schizogony takes place. An optimal temperature of 30°C is necessary for the development of the parasite within the mosquito body; fertilization and penetration of the ookinetes into the stomach wall of the mosquito do not take place at temperatures below 16-17°C. At 25°C sporozoites of *Pl. vivax* develop in 10 days, those of *Pl. falciparum* in 14, and those of *Pl. malariae* in 18 days. At temperatures below 0°C the parasites die in the mosquito body, but at 4-10°C they remain viable. An infected mosquito is capable of transmitting plasmodia for a period of one month. There is no transovarial transmission of the malarial plasmodia in the mosquito. The characteristics of the main species of malarial parasites are presented in Table 33.

Cultivation. The malarial parasites grow on nutrient media which contain blood and glucose.

The differential characteristics of the *Anopheles* and *Culex* mosquitoes, the vectors of the causative agents of malaria, are shown in Fig. 149.

The genus of *Anopheles* mosquito comprises about 50 species which are capable of transmitting malarial plasmodia. The main vector of the parasites in the USSR is *Anopheles maculipennis*.

Pathogenicity for animals. At present about 50 species of malarial plasmodia are known to be responsible for diseases in animals and birds. *Pl. knowlesi*, *Pl. kochi*, *Pl. innui*, *Pl. brasilianum*, etc., are pathogenic for monkeys. *Pl. gallinaceum*, *Pl. fallax*, etc., produce malaria in birds. Both amphibians and reptiles are susceptible to the disease.

Mosquitoes of the *Anopheles*, *Culex*, *Aëdes*, *Mansonia*, and other genera are vectors of malarial plasmodia among animals and birds.

Table 33

Differentiation of Malarial Parasites

Parasite	Developmental cycle (hours)	Young schizonts	Mature schizonts	Merulation stage	Gamonts: Female gamonts	Gamonts: Male gamonts
Pl. vivax (causative agent of tertian malaria)	48	Regular ring forms which occupy $^1/_3$-$^1/_4$ of the erythrocyte diameter	Amoeboid form, highly motile, the pigment accumulates in clumps; erythrocytes are enlarged, stain a pale colour, and are speckled	Mulberry-like form; 12 to 20 merozoites are arranged irregularly and are surrounded by compact clumps of pigment	Round cells; the nucleus is small and occurs eccentrically; the cell entirely occupies the enlarged erythrocyte	Form and size are the same; the nucleus is larger, stains intensively, and occurs in the centre of the cell; the cytoplasm stains poorly
Pl. malariae (causative agent of quartan malaria)	72	Identical to those of *Pl. vivax*	Band-like, poorly motile; the parasite lies across the erythrocyte, its nucleus is stretched out along one edge and the pigment is on the opposite edge; the erythrocytes are of a normal size	Regular rosette form; contains 6 to 8, less frequently, 12 merozoites; the pigment accumulates in clumps	Identical to those of *Pl. vivax*, but are smaller, not exceeding the size of a normal erythrocyte	Identical to those of *Pl. vivax* and not exceeding the size of a normal erythrocyte
Pl. falciparum (causative agent of tropical malaria)	48	Small rings which occupy $^1/_5$-$^1/_7$ of the erythrocyte diameter; an erythrocyte may contain 2 or 3 rings; the schizonts are highly motile	Do not occur in peripheral blood	Multiply in the liver cells; form 6 to 24 merozoites	Crescent-shaped; the nucleus is in the centre and the pigment adheres closely to it	Crescent-shaped; the nucleus is large and occupies more than half the length of the cell; the pigment spreads far beyond the nucleus

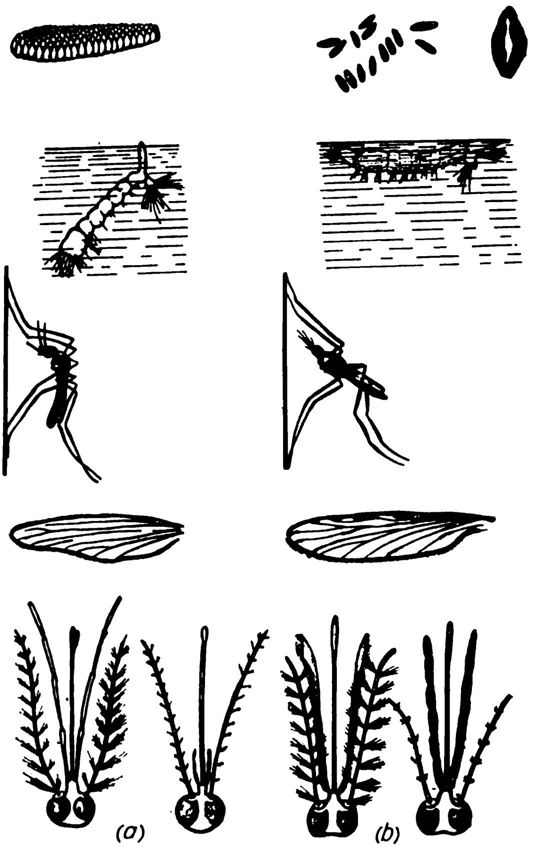


Fig. 149. Differential characteristics of Culex (*a*) and Anopheles (*b*) mosquitoes

The study of malaria in animals and birds has contributed much to the investigation of such important problems as the routes of transmission, pathogenesis, clinical aspects, causes and mechanisms of relapses, diagnosis, treatment, and prophylaxis of malaria in human beings. Certain *Pl. knowlesi* strains which are pathogenic for Macaca monkeys are used for treating (pyrotherapy) progressive paralysis and other diseases.

Pathogenesis and diseases in man. The incubation period in tertian malaria lasts from 10 days to 11 months, in quartan malaria, from 21 to 42 days, and in tropical malaria, from 9 to 16 days. The duration of the incubation period in tertian malaria in northern countries ranges from 9 to 11 months, being 10 months on the aver-

age (*Pl. vivax hibernans*), and in southern countries, from 10 to 18 days (*Pl. vivax vivax*).

A malarial attack is produced as a result of the body reaction to protein substances appearing in the blood due to erythrocyte dissociation. Repeated attacks produce a sensitizing effect and give rise to severe clinical forms of malaria (coma, cerebral oedema). Hyporeactive and areactive forms of malaria exist.

The mechanism of delayed relapses is not completely understood. It is presumed that they depend upon the activization of sporozoites in spring, which persisted in a stable form in the tissue cells.

The disease is accompanied by febrile paroxysms typical for each form of malaria, pathologic blood values, enlargement of the spleen, and development of anaemia. It is also attended by various complications (melanosis of internal organs and tissues, involvement of the liver, kidneys, alimentary tract, nervous system, etc.). Crescent cell anaemia, a menacing and fatal complication, occurs in countries with a high malaria morbidity rate. This condition is due to genetic alterations in the structure of haemoglobin molecules in the erythrocytes, which result in the replacement of glutamic acid by valine in one of the peptide chains. Crescent cell anaemia is caused by homozygosity in relation to one not entirely recessive gene. The altered haemoglobin crystallizes causing destruction of the erythrocytes.

As proved, no chronic form of malaria exists. Tropic malaria lasts for 1 year, tertian, for 18 months, and quartan malaria for as long as 3 years.

Immunity in malaria is associated with humoral and cell factors. Parasitolytic and complement-fixing antibodies are found in patients' blood. Cell factors—phagocytosis of plasmodia by macrophages, i.e., reticuloendothelial cells of the spleen and liver and cells of the reticuloendothelial system in venous sinuses and blood capillaries—play the main role in immunity. A characteristic point is that immunity is conferred only to the given species. For example, an individual who had suffered tertian malaria does not acquire immunity to the tropical form of the disease. Crescent-shaped erythrocytes are responsible for one of the forms of immunity to malaria. This condition is a mild form of anaemia which occurs in heterozygous individuals whose erythrocytes have a tendency to become crescent-shaped and highly resistant to malaria, in particular to the tropical form. The malarial plasmodia are destroyed in these erythrocytes. This condition is widespread among the population of Central Africa. Owing to this, children who had previously suffered from malaria become insusceptible to re-infections at the age of 9-14 years.

Malaria may be accompanied by periods of latent exoerythrocytic parasite carriage. This temporal state of well-being is disturbed under the influence of various external factors (insolation, cooling,

injury, vaccine injections, attendant infection) and relapses of malaria occur.

Laboratory diagnosis comprises microscopy of thick films and smears stained with Romanowsky-Giemsa stain and recognition of the parasite species.

Treatment and prophylaxis. Therapeutic and prophylactic measures are aimed at attacking the source of infection, the malaria patient, who is the carrier and long-term host of the malarial parasites. All malaria patients must be registered as early as possible. Adequate combined treatment with acrichin (quinacrine), plasmocide, quinocide, quinine, bigumal (paludrine), chloridin (pyrimethamine), cycloquine, and resoquine is necessary.

Mass prophylaxis is conducted among persons who have suffered malaria in the previous year. They are given acrichin or bigumal together with plasmocide throughout the season. All malaria patients are kept under observation for at least $1^1/_2$-$2^1/_2$ years.

Measures of control of the malarial vectors, the Anopheles (destruction of the winged mosquitoes in houses), are conducted twice during the season, using insecticides (DDT and hexachlorane).

For larvae control water reservoirs are treated with DDT preparations, and Gambusia, which destroy mosquito larvae and pupae in vast numbers, are cultivated in reservoirs used for water supply.

Malaria prevention includes hydraulic drainage of water reservoirs. Of great importance are precise sanitary supervision during construction of forest-belts, canals, ponds, and reservoirs, and control of prophylactic measures conducted among the builders and population.

Before the Great October Revolution more than 3 million people contracted malaria in Russia annually. The morbidity rate was also high in the years following World War II. Nowadays malaria has been wiped out in the USSR.

In 1955 a total of 200 million of patients with clinical form of malaria was registered throughout the world, and more than 2 million people died from the infection. There is a high prevalence of malaria in India, Pakistan, Ceylon, Burma, tropical Africa, and East Africa.

BALANTIDIA

The microorganism *Balantidium coli* is the only infusorial parasite of man, which belongs to the class *Ciliata*, family *Bursaridae* and is responsible for human balantidiasis. It was discovered in 1856 by the Swedish physician P. Malmsten.

Morphology. The balantidia range from 50 to 70 μ in length and from 40 to 70 μ in breadth (Fig. 150). Sometimes they are 200 μ long and 70 μ broad.

The parasite has an asymmetrical oval body covered with cilia.

Its anterior end is more pointed than the posterior end and has a mouth aperture (cytostome) which leads into a short oesophagus. Large cilia arranged close to the cytostome form a peristome which directs food into the oesophagus together with a flow of fluid. The posterior end of the body has an anal pore, the cytoproct. A thin layer of alveolar ectoplasm lies beneath the pellicula (membrane). A kidney-shaped macronucleus containing chromatin bodies and

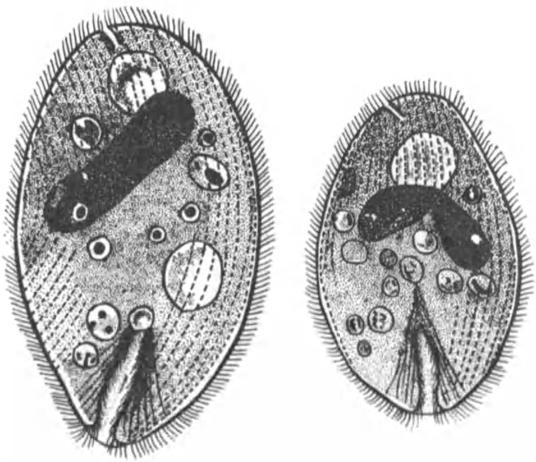


Fig. 150. Balantidia of the human intestines

several nucleoli lies submerged in the granular endoplasm. The micronucleus is situated on the concave surface, and there are two contractile vacuoli. The parasites multiply by horizontal fission.

Within the human intestines the parasite is encysted in a double-layer membrane, losing its covering of cilia. The cysts are from 30 to 60 μ in diameter.

Cultivation. The organism is cultivated on special media (meat-peptone broth diluted in a ratio of 1:5 in a 0.85 per cent common salt solution and 10 per cent horse serum and containing a loopful of rice starch; pH 7.4). By adding antibiotics to the medium balantidia cultures which are free of bacterial microflora can be maintained.

Pathogenicity for animals. Domestic and wild swine, monkeys, grey rats and other animals are susceptible to balantidiasis. Swine and grey rats play a major part in the epidemiology of this disease, infecting human beings.

Pathogenesis and disease in man. Balantidia cause lesions in the colon, producing ulcers, abscesses, and colitis characterized by the presence of blood and mucus in the stool. The disease is accompanied

by loss of appetite, headache, and emaciation. One third of all cases terminate in death. Asymptomatic carrier states have also been registered.

The infection is spread by swine which carry the parasite in their intestines. The parasite enters the human body mainly in the encysted state. However, penetration of the motile (vegetative) forms of balantidia is not excluded since they are highly resistant and may withstand the action of gastric juice for 12 hours. The parasite may invade the blood and lymph vessels and the muscular coat of the stomach.

Immunity in balantidiasis is not known.

Laboratory diagnosis. Fresh stools are examined. Live infusoria are clearly seen in unfixed smears under the microscope in the form of actively moving large cells. Cysts are seldom formed in the human body, and if so, only in small numbers, and are of no diagnostic value.

Treatment. The same drugs as in amoebiasis are used. In addition, quinine nitrate or silver nitrate enemas are prescribed. Osarsol (acetarsone), hexylresorcinol, and chlortetracycline are administered orally.

Prophylaxis is ensured by sanitary-hygienic measures (protection of foodstuffs and water from contamination with swine faeces and observation of individual hygiene when taking care of the animals).

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He has published *Lectures on General and Special Microbiology*, *Handbook of Practical Work in Medical Microbiology*, and other works for students of medical institutes.

In the Soviet Union his textbook has been reprinted twice. It has also been published in Spanish